PHD

Synthetic studies of (±)-anatoxin-a related to its use in affinity chromatography

Huby, Nicholas John Silvester

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Synthetic Studies of (±)-Anatoxin-a Related to It's Use in Affinity Chromatography

submitted by Nicholas John Silvester Huby

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Dedicated to my parents,

for their unswerving support and love

over the years.

ABSTRACT

This thesis is introduced by a brief review of some of the biospecific affinity techniques which have been used in the characterisation of the neuromuscular nicotinic acetylcholine receptor. Affinity chromatography, affinity partitioning and photoaffinity labelling are described along with the use of various agonists and antagonists in these techniques. (+)-Anatoxin-a appears well suited for the study of the nicotinic acetylcholine receptor in brain membrane.

Further to the previously reported synthesis of (±)-anatoxin-a, using as a key step the stereoselective silver(I) mediated cyclisation of a secondary sulphonamide onto an allene, it is shown that an efficient enzymatic resolution of the cyclisation precursor can be effected with α-chymotrypsin. The resolved cyclisation precursor is shown to be of high optical purity by ^{1}H n.m.r. studies and to be the correct absolute configuration for (+)-anatoxin-a synthesis by correlation with D-glutamic acid. Using the same cyclisation precursor in racemic form it is possible to form an α,β-unsaturated ketone which is of potential use in affinity ligand preparation.

Adapting the procedure of Lindgren *et al.* for the synthesis of (±)-anatoxin-a it is possible to introduce various substituents, in place of the C-11 methyl group, via the regiospecific alkylation of an allylic anion. Elaboration of these C-11 substituents, to make the molecule suitable for immobilisation on a polymeric support, was investigated. An ortho ester derivative was found to be unstable to the conditions employed for elaboration whilst benzyl halides could not be converted to the corresponding methyl esters under palladium (0) catalysis. It is shown that a terminal alkene substituent at C-11 can be converted to a methyl ester by ozonolysis

and oxidation of the resulting aldehyde after protection of the nitrogen as a *t*-butyl carbamate. Deprotection of the bicyclic amine yields a suitable model of an affinity ligand based on (±)-anatoxin-a which is found to bind with high specificity and affinity to the nicotinic acetylcholine receptor in rat brain.

The alkylation of the kinetic enolate of a protected form of (±)-anatoxin-a is reported. Deprotection yields a compound which is a model of a potential high affinity and specificity radiolabelled ligand for the nicotinic acetylcholine receptor.

Contents

Abbreviations

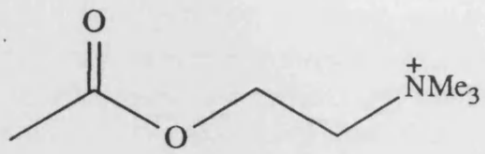
ANB-AI	*N*-5-Azido-2-nitrobenzoylaminoacetimidate hydrochloride
Anatoxin	(+)-Anatoxin-a
Boc	(2-Methylpropan-2-yl)-oxycarbonyl
Bis-Q	3,3′-Bis(α-trimethylammonium)methyl-azobenzene
CNS	Central nervous system
DDF	4-(Dimethylamino)benzenediazonium tetrafluoroborate
DME	1,2-Dimethoxyethane
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulphoxide
EDTA	Ethylenediaminetetraacetic acid
HMPA	Hexamethylphosphoric triamide
HOMO	Highest occuppied molecular orbital
HSAB	Hard and soft acids and bases
LDA	Lithium diisopropylamide
LUMO	Lowest unoccuppied molecular orbital
nAChR	Nicotinic acetylcholine receptor
NAD	Nicotinamide adenine dinucleotide
n.m.r.	Nuclear magnetic resonance
PCC	Pyridinium chlorochromate
t-butyl	2-Methylpropan-2-yl
THF	Tetrahydrofuran
t.l.c.	Thin layer chromatography
Triflate	Trifluoromethane sulphonate
Ts	Tosyl, 4-(methylbenzene)sulphonyl

To travel hopefully is a better thing than to arrive,

and the true success is to labour.

(VI El Dorado, R. L. Stevenson)

1. Introduction



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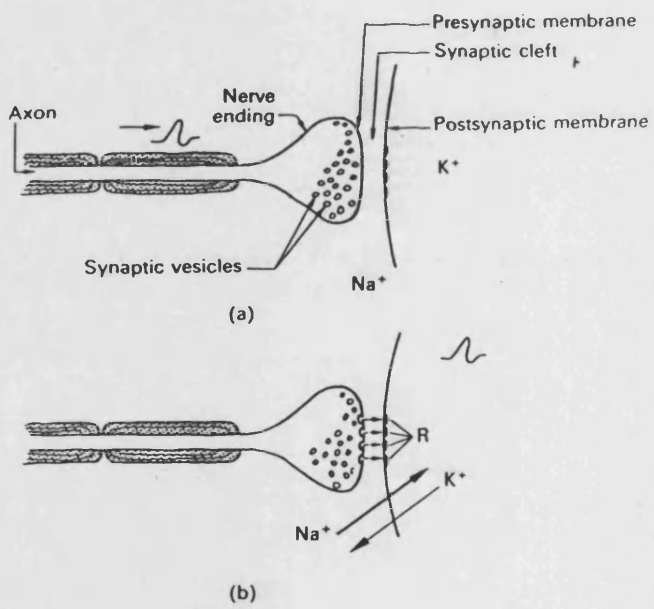


(∿) = Action potential

Figure 1 : Schematic drawing of synaptic transmission.

In 1906, Langley[1] concluded his experiments on the effect of nicotine on muscle contraction in the fowl by postulating that a "receptive substance...combines with nicotine and curare and is not identical with the substance that contracts." This observation was followed by a series of important experiments in the laboratories of Loewi and Dale which resulted in their sharing of the 1936 Nobel prize. Loewi[2] developed the concept of neurotransmitter function, while Dale[3] and co-workers provided specific indication for the role of acetylcholine **1** as a neurotransmitter in the mammalian system. It is on their work that our basic concept of cholinergic synaptic transmission is based.

A working hypothesis for the series of events involved in synaptic transmission has been developed (figure 1). When an invading action potential arrives at the pre-synaptic nerve terminal the resulting depolarisation (the membrane potential becoming more positive than the resting potential of -70 mV) increases the probability of transmitter release from synaptic vesicles into the cleft. This is thought to happen in one of two ways: (1) that the whole vesicle passes into the cleft where it disintegrates, discharging the acetylcholine it contains (exocytosis), or (2) that the vesicle on coming into contact with the pre-synaptic membrane, opens up into a pore in the synaptic membrane through which the transmitter passes. The acetylcholine in the synaptic cleft either: (1) diffuses out of the cleft; (2) is hydrolysed by acetylcholine esterase; or (3) binds to a receptor on the post-synaptic membrane. The post-synaptic acetylcholine receptor then undergoes a conformational change leading to the opening of ionic channels, thereby increasing the conductance of the post-synaptic membrane. Sodium ions flow in and potassium ions flow out of the nerve cell down their respective electrochemical gradients. To commence with the influx of sodium ions is greatest and so the membrane potential slowly increases from the resting potential of -70 mV up to -60 mV. There is then a rapid overshoot to the extent that the membrane potential may become positive, to

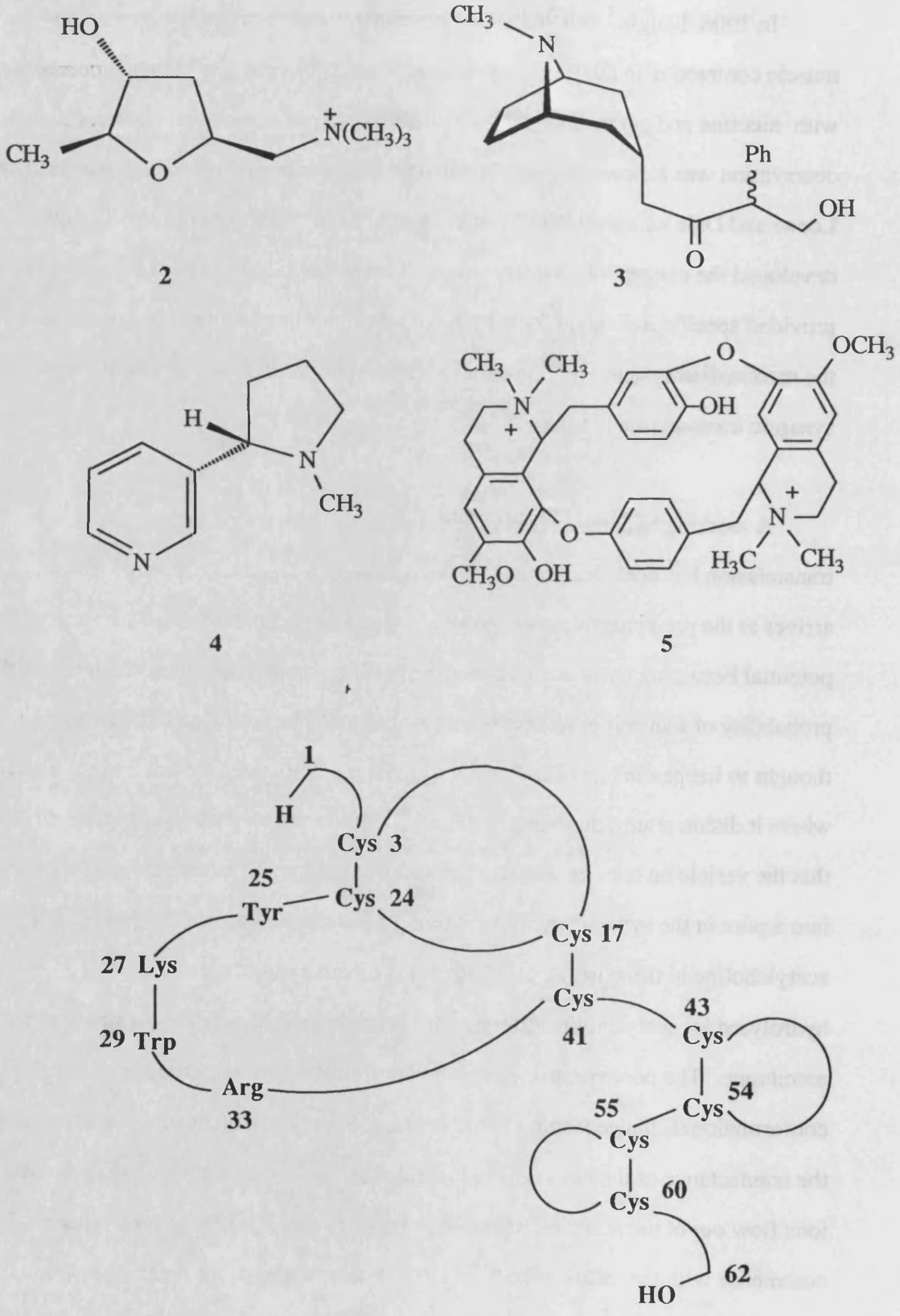


Figure 2 : Homologous amino acid residues in snake α-toxins.

some +10 mV to +30 mV, known as the action potential. The action potential then proceeds down the next nerve axon.

Two types of cholinergic receptor have been determined pharmacologically: "muscarinic" where neurotransmission is mimicked by muscarine **2** and blocked by atropine **3**; "nicotinic" where neurotransmission is mimicked by nicotine **4** and blocked by *d*-tubocurarine **5**. The nicotinic receptor for acetylcholine (nAChR) is now the best understood neurotransmitter receptor. This is due to two factors: (1) the availability of a rich source of analogous tissue from the electric organs of *Electrophorus* and *Torpedo* species; (2) the availability of small peptide α-toxins from snake venoms which are potent and essentially irreversible antagonists of neuromuscular transmission.

The two main families of snakes whose venoms have found use in this field are the *Elapidae* (cobra, krait, coral snake, mamba, *etc.*) and *Hydrophidae* (many species of sea snake) who produce the *Naga naga* α-neurotoxins.[4] Erabutoxin-b, obtained from *Laticauda semifasciata* and which behaves pharmacologically in the same way, has also been used. Cobra and sea snake neurotoxins can be grouped into two families of homologous peptides according to the number of amino acid residues present. Neurotoxins belonging to the first group are composed of 61 or 62 residues of 15 or 16 amino acids in a single peptide chain crosslinked by 4 disulphide bonds. The second group is composed of 71 residues of 17 or 18 amino acids with 5 disulphide bonds. The majority of cobra and sea snake neurotoxins so far isolated belong to the first group. They display a high degree of structural similarity with the position of disulphide bonds being highly conserved (figure 2). Reduction of all the disulphide bonds, to give a linear peptide, results in a complete loss in toxicity.

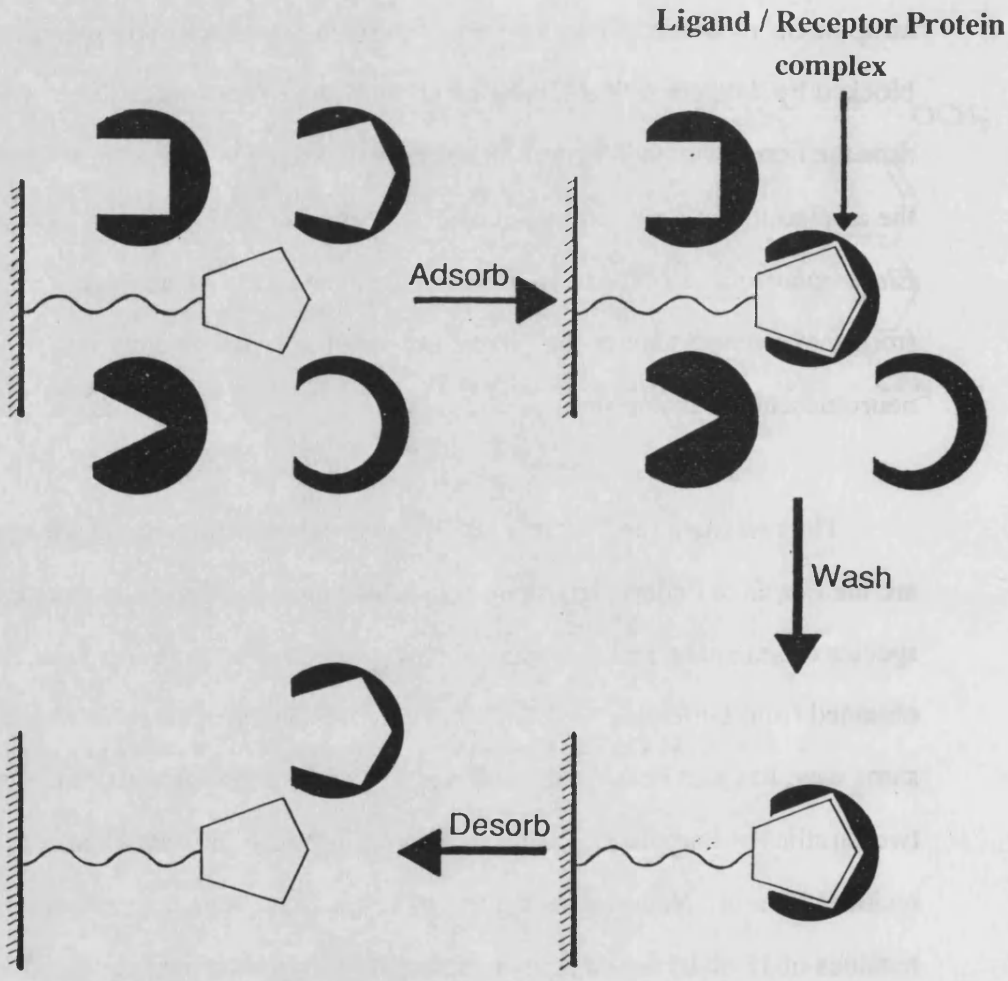
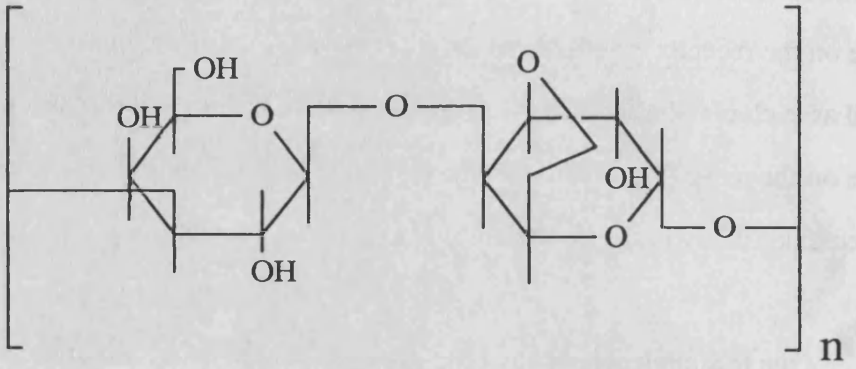


Figure 3 : Schematic representation of affinity chromatography.

The mode of action of cobra and sea snake neurotoxins is to produce a neuromuscular blockade at the postsynaptic receptor thereby inhibiting depolarisation. In particular these toxins have been used because they are essentially irreversible antagonists *i.e.* they do not dissociate readily once bound to the receptor protein, and are essentially identical in action with *d*-tubocurarine **5**. However, it should be noted that whilst these neurotoxins are binding at the same site on the receptor protein as an agonist they are eliciting a complementary effect and as such may be acting by a different mode of action, *e.g.* through a neighbouring site on the receptor protein. Despite this complicating factor these toxins have been used extensively in the localisation, isolation and characterisation of the nAChR.

One technique which has been of particular use in the isolation and characterisation of receptor proteins is affinity chromatography.[5] The principles of this technique can be dated back to 1910 when there were reports of the isolation of amylase by adsorption to insoluble starch.[6] The power of affinity chromatography lies in its highly selective "insolubilisation" of macromolecules from solution. Purification is achieved by chromatography of a mixture containing the protein to be purified on a column of an insoluble matrix to which a specific ligand has been bound (figure 3). Proteins and other materials not exhibiting appreciable affinity for the ligand will pass unretarded through the column, whereas those that bind to the ligand will be retarded. Elution of the bound protein is achieved by changing the mobile phase such that conditions are unfavourable for combination.

As a separation tool it is unique in that its application requires a good deal of knowledge of the biochemical system in which it is to be used before the affinity adsorbent can be designed. The problem of selecting the matrix and the ligand reoccurs with each new system approached. A useful ligand will have a high affinity ($K_D \geq 10^{-5}$) for the molecule that is to be purified. Having such a ligand, a



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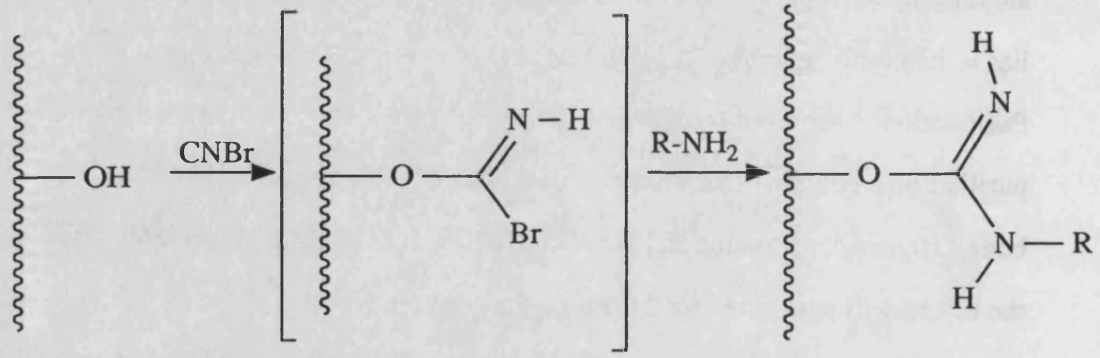


Figure 4 : Activation of agarose with cyanogen bromide for coupling of an amine.

point of chemical modification must be available. The aim is to retain the biological binding ability of the ligand after it is attached to the matrix. The effect of chemical modification of the potential ligand can be studied with soluble model systems. A soluble derivative can for instance be assayed for its binding affinity. Tests of this type may show that the modified ligand has an enhanced affinity, this has been ascribed to hydrophobic contributions to binding from the presence of the spacer-arm.

The insoluble matrix typically used is a commercially available polysaccharide matrix *e.g.* agarose, which is both chemically and biologically inert under most conditions. This sort of matrix is made up of the repeating D-galactose/3,6-anhydro-L-galactose sub-unit **6**. Attachment of the ligand is achieved by prior activation of the matrix with cyanogen bromide (figure 4).[7] A ligand bearing an amine substituent will then bind to the activated matrix to form an *N*-substituted *iso*urea.

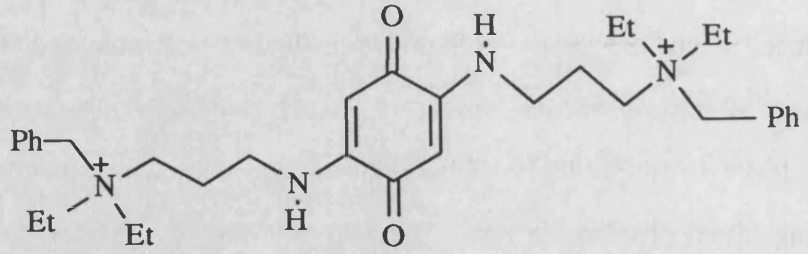
Affinity chromatography using purified snake venoms as the ligand has been used successfully in the isolation of nAChR from vertebrate muscle. Conventional biochemical techniques that were initially applied to the purification of the nAChR met with only limited success. This was attributed to low levels of purification and receptor desensitization. The use of affinity chromatography in the purification of the nAChR was made possible by the discovery that it was possible to solubilise the receptor protein using mild, non-ionic detergents without loss of the ability to bind cholinergic agonists and antagonists.[8]

In 1973 the purification of the nAChR from the electric organ of the electric eel *Electrophorus electricus* was reported,[9] using an affinity column containing the purified venom of the Thailand cobra, *Naga naga siamensis*. The agarose column

was activated with cyanogen bromide and used to bind the cobra neurotoxin along with a small amount of tritium labelled neurotoxin. The tritium labelled neurotoxin was included so that the amount of bound toxin could be assessed, it was found to be in the range 90-95%. However, based on the receptor binding capacity of the material, less than 10% of the covalently bound neurotoxin was capable of combining with the receptor protein. This was obviously a reflection the non-specific method of attachment of the neurotoxin to the matrix. Despite this limitation it was possible to isolate a 5% yield of material which exhibited 8250-fold increased cholinergic activity.

Using a slightly different method of affinity chromatography Raftery[10] was able to purify the nAChR from *Torpedo Californica* electroplax membranes using an affinity column with attached snake venom. The neurotoxin was isolated from the venom of the Formosan cobra, *Naga naga atra*, and attached to the column after cyanogen bromide activation. The nAChR was absorbed onto this column when the homogenous extract of torpedo electroplax containing a mild detergent was passed through the column. The neurotoxin used formed a strong but reversible complex with the nAChR. Therefore, the receptor protein could be obtained by displacement with a solution of the more strongly complexing (lower K_D) toxin α-bungarotoxin. By this method a major protein sub-unit was found which had an estimated relative molecular mass of $3.5\text{-}4.5 \times 10^4$ by gel electrophoresis under denaturing conditions.

Affinity chromatography to purify the nAChR from torpedo electroplax using the purified toxin from *Naga naga siamensis* has been reported[11] to give different results depending on what eluant was used to desorb the bound toxin. The nAChR studied was that obtained from *Torpedo marmorata* after homogenisation and solubilisation with Triton X100 (a detergent). When desorption was performed with 10mM benzoquinonium **7** a protein was obtained which could be labelled with



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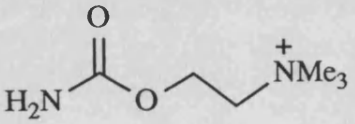

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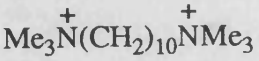

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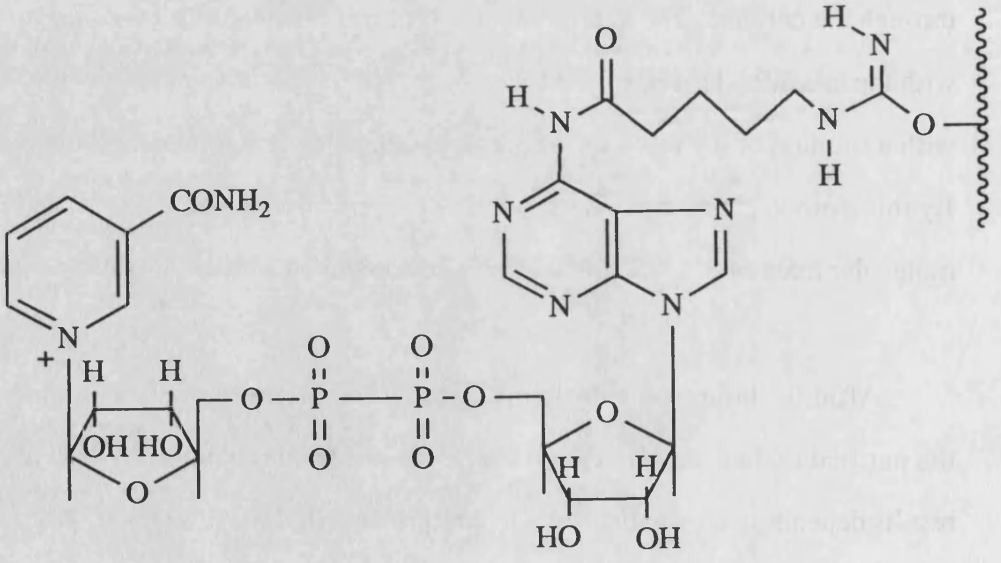

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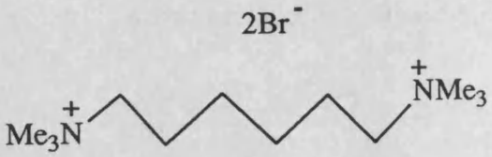
[^{125}I]α-bungarotoxin but not with [^{3}H]acetylcholine or other reversible cholinergic ligands. However, when the affinity column was eluted with 1M carbamylcholine **8** a nAChR protein was obtained which was found to bind [^{3}H]acetylcholine, [^{3}H]decamethonium **9**, [^{3}H]nicotine, [^{14}C]dimethyl-*d*-tubocurarine and [^{125}I]α-bungarotoxin.

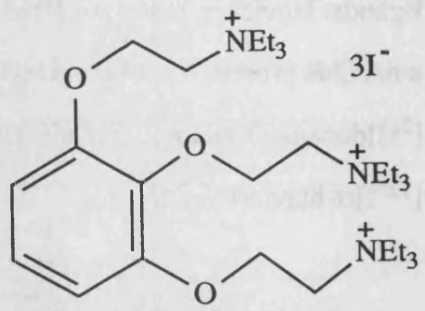
Another complicating factor to be contended with in the isolation of the nAChR by affinity chromatography is the possibility of isolating acetylcholine esterase. *In vivo* acetylcholine binds reversibly at the corresponding postsynaptic receptor, to cause depolarisation, and is hydrolysed to choline within the synaptic cleft by acetylcholine esterase. The two receptor sites involved may therefore be sufficiently similar that an affinity column may bind both the postsynaptic receptor protein and the enzyme. Fortunately, it was found with snake α-toxins that they demonstrate a high selectivity for the postsynaptic nAChR. This was only known after assaying the purified receptor for acetylcholine esterase activity.

Although non-specific adsorption (due to the hydrophobic effect of the spacer-arm) may be problematic it can also be used to advantage as an initial purification step for isolation of a particular class of compounds. By suitable choice of eluant it is then possible to selectively desorb each particular sub-class of molecules. According to this ethos, Lowe and coworkers[12] used nicotinamide adenine dinucleotide (NAD) immobilized onto 6-aminohexanoyl substituted agarose **10** for the purification of NAD-dependant dehydrogenases from crude extracts. Specific desorption was achieved by gradient elution with an increasing concentration of potassium chloride. Selectivity for different classes of enzymes could be achieved by using different immobilised co-factors as the affinity ligand.

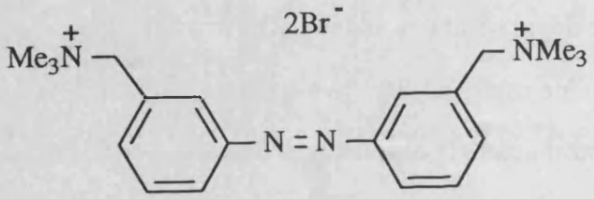
Apart from snake venoms the other major class of compounds which have


2Br-
Me3N+
NMe3+

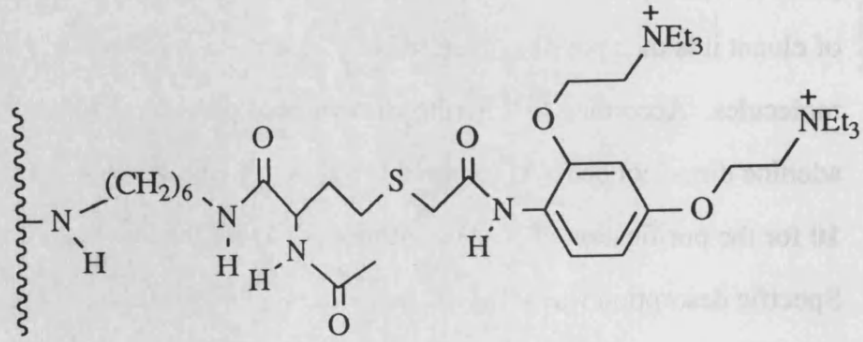
11


NEt3+
3I-
O
O
NEt3+
NEt3+
O

12


2Br-
Me3N+
NMe3+
N = N

13


NEt3+
NEt3+
O
O
O
(CH2)6
N
H
N
H
O
S
N
H
N
H
O
O

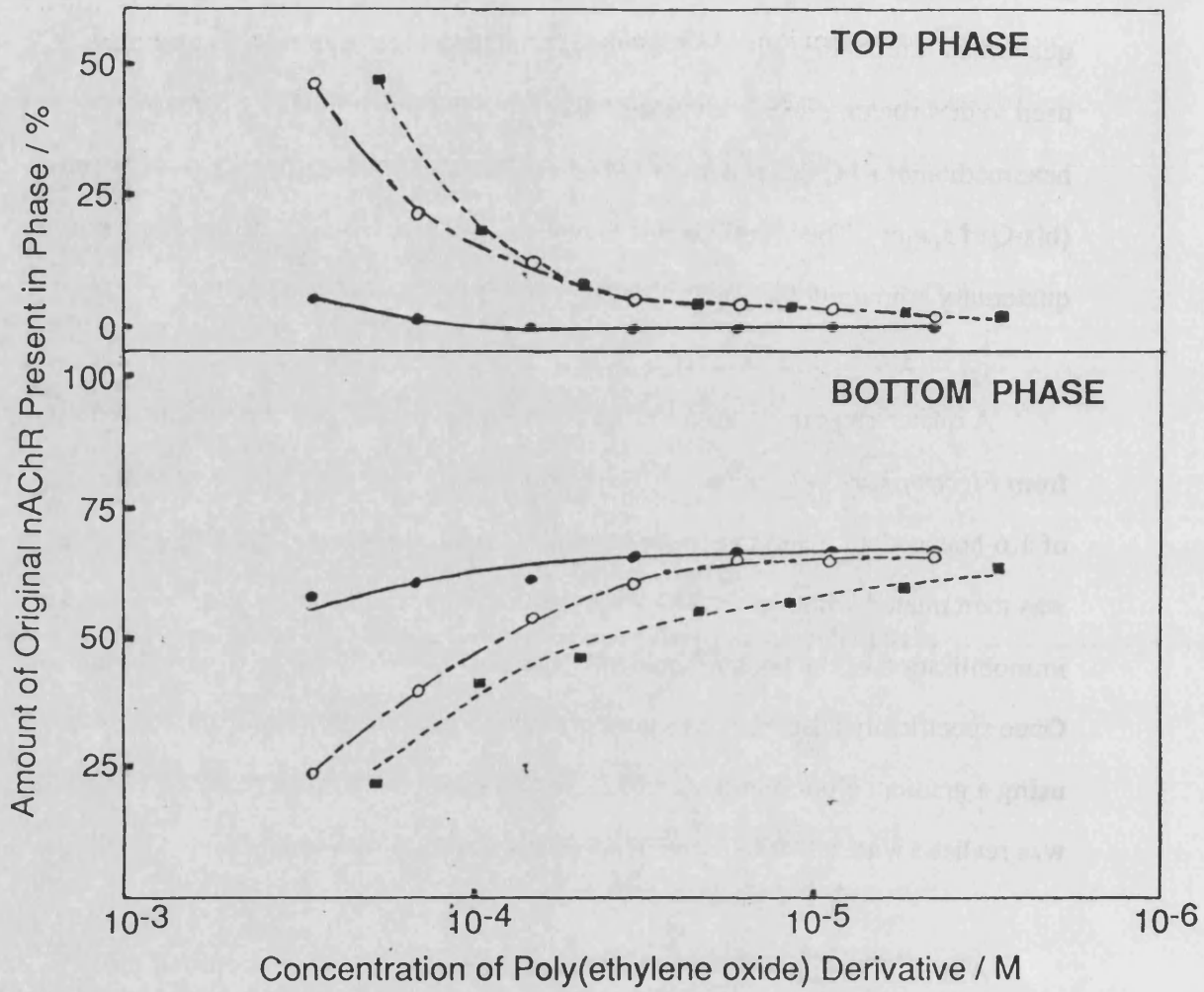
14

been used as affinity ligands for the purification of the neuromuscular nAChR are quaternary ammonium ions. Compounds containing these groups are also often used to desorb complexed receptor protein from α-toxin columns *e.g.* the use of hexamethonium **11**, flaxedil **12**, 3,3′-bis(α-trimethylammonium)methyl-azobenzene (bis-Q) **13**, *etc.*. These compounds presumably display nAChR affinity because the quaternary ammonium group mimics that found in acetylcholine **1**.

A quaternary ammonium ion was used in the isolation of the nAChR protein from *Electrophorus electricus*.[13] The affinity column was prepared by the addition of 1,6-hexanediamine to cyanogen bromide activated agarose. The amino-agarose was then treated with *N*-acetyl homocysteine thiolactone followed by immobilisation of the bis-ammonium salt to give the immobilised affinity ligand **14**. Once specifically adsorbed onto this column the receptor protein could be eluted off using a gradient elution of flaxedil **12**. In this way a 30-50% recovery of nAChR was realised with a 150-fold purification of the receptor protein.

Although the attainment of highly purified receptor proteins from these affinity columns[14] may argue well for the specificity of the interaction, the concentrations of the eluting ligands used was often in the millimolar range for antagonists and in the molar range for agonists such as carbamylcholine **8**. This may imply that desorption is non-specific in nature with several sub-classes of nAChR being eluted simultaneously. An exception to this is the study by Chang[15] on the purification of the nAChR from *Electrophorus electricus*. Chang utilised a phenyl- trimethylammonium affinity ligand and eluted the receptor protein with 3μM bis-Q **13**.

In addition to cholinergic ligands, Raftery and coworkers[16] have used sodium chloride gradients up to 0.5M to elute receptor proteins from quaternary ammonium



$$R\text{-}(CH_2\text{-}CH_2\text{-}O)_{156}\text{-}CH_2\text{-}CH_2\text{-}R$$

15 $R = -\overset{+}{N}(CH_3)_3$

16 $R = -\overset{H}{\overset{|}{N}}-C_6H_4-\overset{+}{N}(CH_3)_3$

17 $R = -\overset{+}{N}(CH_3)H_2$

Figure 5 : Affect of quaternary ammonium ligands in affinity partitioning of nAChR containing membranes.

affinity columns. The fact that common salt displaces the nAChR in similar concentrations to some cholinergic ligands suggests that the affinity step may not be all that specific. The sodium chloride elution method only works with quaternary ammonium substituted affinity columns and not α-toxin substituted columns.

The purification of the nAChR by affinity chromatography is indicative of a specific receptor-ligand interaction. However, in these studies the receptor proteins are solubilised with detergent and not membrane bound as they would be *in vivo*. More convincing evidence for interaction of immobilised cholinergic ligands with the membrane-bound nAChR comes from a technique known as affinity phase partitioning.

Affinity phase partitioning is an adaptation of phase partitioning. In phase partitioning a distribution or partitioning of substances into two phases, obtained by mixing aqueous solutions of two different polymers, can be obtained as a function of polymer charge, ionic strength, pH and hydrophobic character of the polymers. A typical system employs high molecular weight dextran and poly(ethylene oxide) which separate into two phases above a certain "critical" concentration. Macromolecules, membranes or cells will partition into either the dextran rich or poly(ethylene oxide) rich phase as a function of the parameters given above. In affinity phase partitioning additional selectivity is obtained by the covalent attachment of an affinity ligand to one of the polymers introduced into the system.

In studies performed by Flanagan, Barondes and Taylor[17]quaternary ammonium salts bis-α,ω-trimethylammonium poly(ethylene oxide) **15** or bis-4-trimethylammoniumphenylamino poly(ethylene oxide) **16** were used as cholinergic ligands. In the absence of these ligands it was found that membranes from the electric organs of *Torpedo Californica* partitioned such that 1% of the

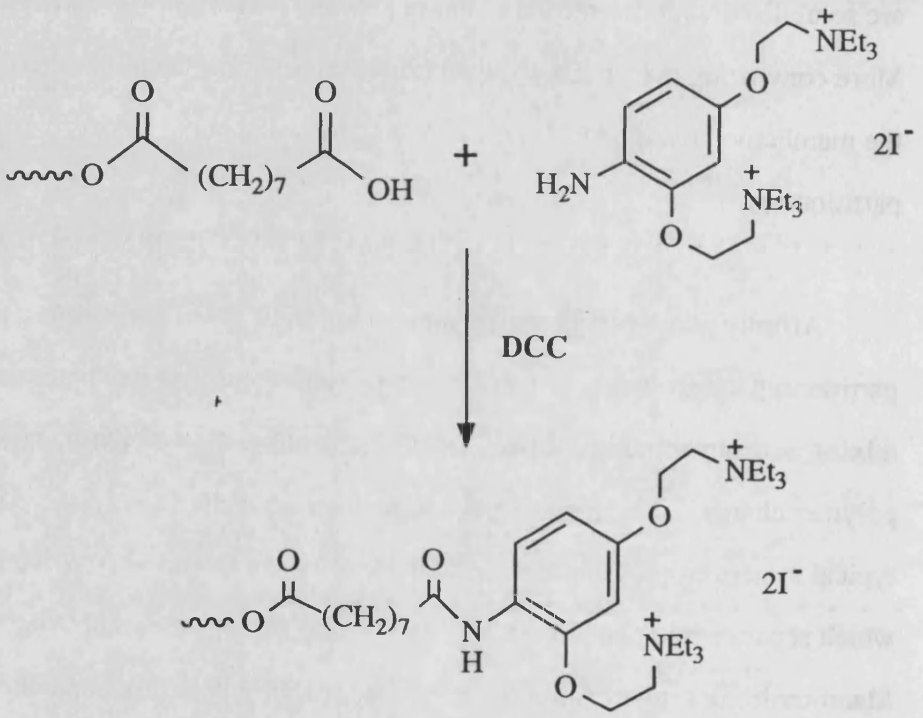


Figure 6 : Preparation of quaternary ammonium affinity ligand with azelaic acid spacer-arm.

membranes containing the nAChR were in the poly(ethylene oxide) rich top layer and 65% in the dextran rich bottom layer. The remaining material was presumably localised at the phase interface. When a small amount of poly(ethylene oxide) was replaced by either of the quaternary ammonium salts there was a large increase in the concentration of nAChR containing membrane in the poly(ethylene oxide) rich phase (figure 5). To show that this was due to specific binding to the nAChR the experiment was repeated using bis-α,ω-methylamino poly(ethylene oxide) **17**, which should have been protonated at the pH of the experiment and which should impart an equivalent interfacial electromotive potential to the system. With this ligand only a very small change in phase distribution was measured.

More recently[18] the effectiveness of this technique has been improved by the use of a different bis-quaternary ammonium ligand. 1,3-Bis(2-triethylammonium ethoxy)-4-aminobenzene diiodide was attached to a poly(ethylene oxide) polymer via a spacer-arm of azelaic acid (figure 6). Using this ligand at 0.4% concentration in poly(ethylene oxide) with dextran as the second phase it was found that 94% of the cholinergic receptor sites concentrated in the poly(ethylene oxide) rich phase.

Another biospecific affinity technique related to affinity chromatography which is suitable for use with membrane-bound proteins is photoaffinity labelling.[19] This technique has been used extensively in the isolation and characterisation of transmitter receptor proteins. In photoaffinity labelling the ligand (which often contains a radioactive marker) becomes covalently bound to the receptor when a functional group is photoactivated by ultraviolet or short-wave visible light. A major advantage of this technique is that the photoactivation may be done at a time of the experimenter's choosing. Prior to photoactivation an equilibrium is set up between ligand and receptor, therefore placing some of the ligand at the receptor binding site.[20] Photoactivation of the ligand produces a reactive intermediate which

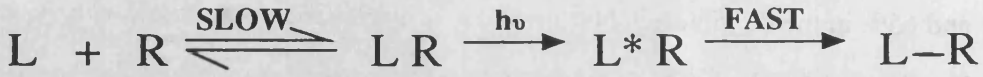


Figure 7 : Steps involved in photoaffinity labelling.

L = photolabel , **R** = receptor.

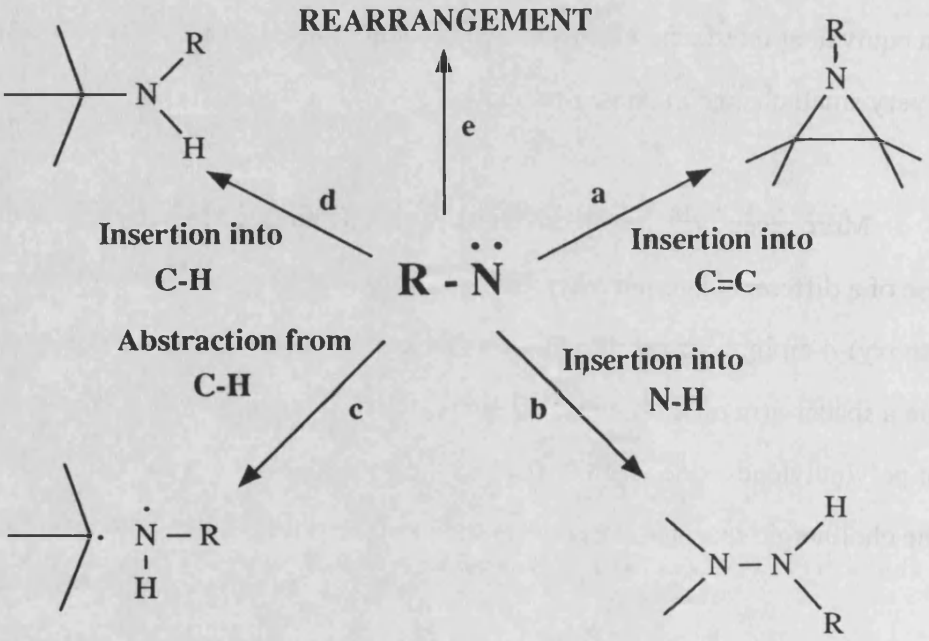
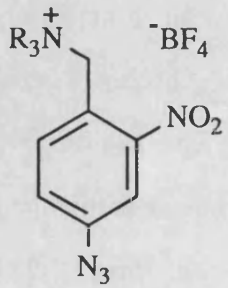


Figure 8 : Reaction pathways for nitrene photolabel.

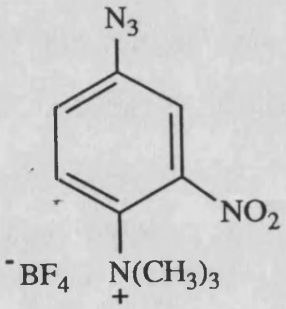


18 R = Et
19 R = Me

rapidly becomes covalently bound to its binding site (figure 7). For the technique to be effective the unbound ligand on activation must react more rapidly with the surrounding solvent than with some other part of the receptor protein.

The most commonly used photolabile groups have been aryl azides which were introduced by Fleet, Porter and Knowles[21] for the affinity labelling of antibodies. On photolysis nitrogen is lost to form very reactive, electron deficient aryl nitrenes. Due to their extreme instability they can undergo a number of different reactions (figure 8). This is advantageous if the receptor site is barren of functional groups that will react with an alternative labelling reagent. However, only some of the reactions will lead to a ligand-receptor site covalent bond (paths a, b and d, figure 8). With some substituents R, rearrangement (path e) will predominate. Therefore, alkyl nitrenes are usually found to be inefficient as photoaffinity labels. Acyl nitrenes are also unsuitable in this respect as they can undergo the Curtius rearrangement to an isocyanate. Also, photolysis is only effective at wavelengths so short (300nm) that aromatic residues in the protein would be destroyed by the incident radiation. Aryl nitrenes are the least susceptible to rearrangement, with appropriate substituents their adsorption maxima are well clear of protein adsorptions and they give the highest efficiencies, though on average this is still only in the region of 10%. Due to the high reactivity of nitrenes they are usually non-specific in there reactions and a number of amino acid residues in the vicinity of the receptor site can be labelled.

In 1970 the aryl azides **18** and **19** were used in the photoaffinity labelling of the membrane-bound nAChR in frog sartorius muscle.[22] Neither compound produced any depolarisation of the muscle membrane without photolysis but behaved as antagonists to depolarisation by carbamylcholine **8**. Photolysis of the muscle bathed in solutions of the aryl azides **18** and **19** caused irreversible losses in



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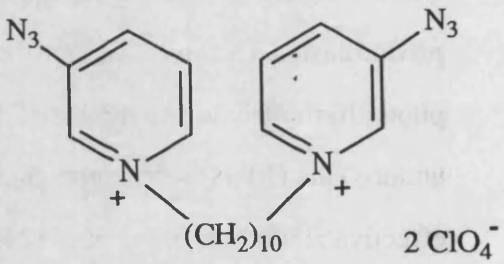

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receptor activity, both compounds being equally effective. The muscle lost none of its activity when photolysed in the absence of the aryl azides or in the presence of the aryl azide photolysis products, formed independently. The loss in activity could be prevented by carrying out the experiment in the presence of *d*-tubocurarine **5** which blockades the nAChR. Photolysis of the muscle protected in this way followed by washing to remove *d*-tubocurarine, afforded tissue which retained 91% of its activity.

Photoaffinity labelling of the nAChR has been carried out using photosensitive derivatives of acetylcholine analogues. Hucho and coworkers[23] used as photoaffinity label the tritium labelled acetylcholine agonist 4-azido-2-nitrobenzyltrimethylammonium fluoroborate **20**. It was found with this reagent that labelling was non-specific, four different peptide chains being labelled. However, in the presence of the purified α-toxin from *Naga naga siamensis* no radioactivity incorporation was observed, indicating that the neurotoxin acted as a competitive antagonist.

Witzemann and Raftery[24] used bis(3-azidopyridinium)-1,10-perchlorate **21** as their photoaffinity label. Once again it was found that using a quaternary ammonium affinity ligand labelling was not completely specific. However, the majority of labelling was found to occur to one peptide chain. The remaining non-specific labelling was attributed to : (1) the proximity of ligand binding sites on other peptide chains; (2) alterations in the receptor site topography between membrane-bound and insolubilised states.

As with affinity chromatography, snake α-toxins have been used in photoaffinity labelling studies. The introduction of the photolabile groups has generally been carried out in a less than specific manner. The purified α-toxin from

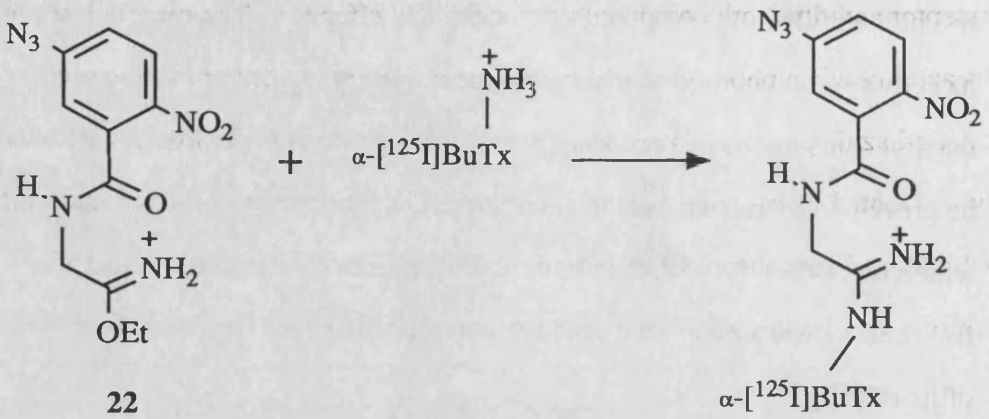

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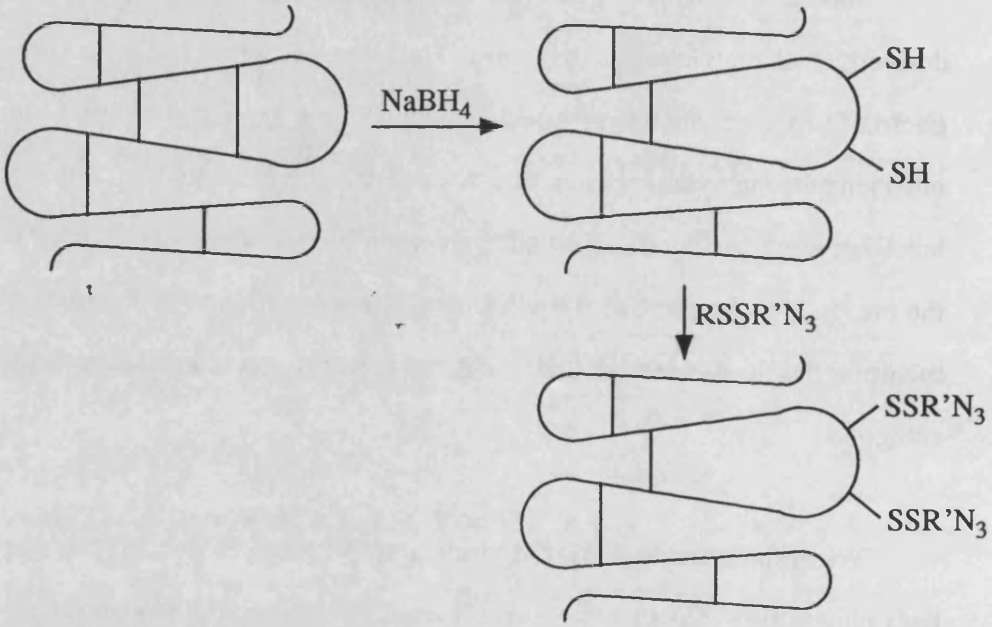
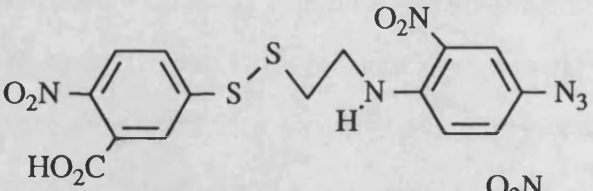
Figure 9 : Derivatisation of α-bungarotoxin for photoaffinity labelling.



25 OR 26

23 RSSR'N3 =



24 RSSR'N3 =

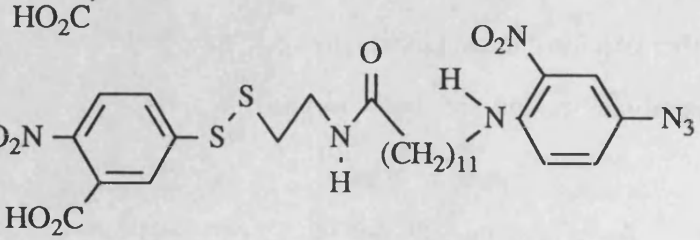
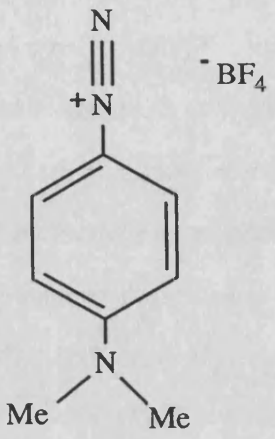


Figure 10 : Controlled reduction and mixed disulphide formation with snake α-toxin.

Naga naga siamensis has been derivatised using a 3-fold excess of 4-fluoro-3-nitrophenylazide in the presence of triethylamine.[25] Four labelled α-toxins were isolated under these conditions, due to the introduction of the label at different positions in the amino acid sequence or by introduction of more than one label. The mixture of labelled α-toxins was found on photolysis not to label any of the protein sub-units previously found for the nAChR. This was thought to have arisen because the size of the α-toxin meant that it extended to areas beyond the nAChR.

Another method of non-specific photoaffinity label introduction which has been reported[26] used ethyl *N*-5-azido-2-nitrobenzoylaminoacetimidate hydrochloride **22** (ANB-AI). Prior to attachment the α-toxin was labelled with radioactive iodine. Treatment of the ^{125}I-labelled α-toxin with ANB-AI liberated ethanol and attached the photolabel to a primary amine group in the α-toxin *e.g.* a lysine residue (figure 9). However, it was found that the labelling of the peptide sub-units of the nAChR obtained from *Torpedo* electric organs was nonspecific with this reagent, presumably due to the same reasons as given above.

Snake α-toxins have also been labelled more specifically.[27] One of the five disulphide bonds found in [^{125}I]α-bungarotoxin can be reduced to a disulphide with sodium borohydride (figure 10). Mixed disulphide formation between the di-thiol and the disulphide reagents **23** or **24** gave the bis-arylazide substituted α-bungarotoxin derivatives **25** and **26** respectively. Using the bis-substituted α-toxin **25**, with the shorter spacer-arm ($\approx 14\times10^{-10}$Å), led to mainly intramolecular cross-linking of a sub-unit with a relative molecular mass of 40,000, with a small amount of cross-linking to a sub-unit with a relative molecular mass of 65,000. Using the derivative **26**, with the longer spacer-arm ($\approx 33\times10^{-10}$Å), gave selectively cross-linking to the 65,000 sub-unit.



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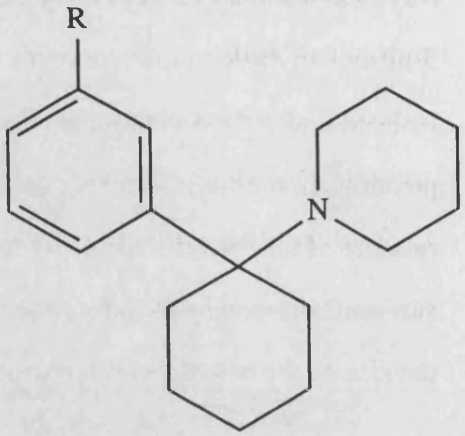
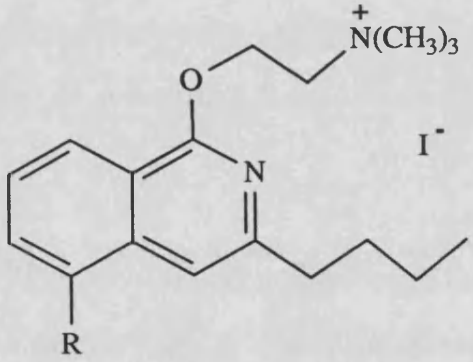


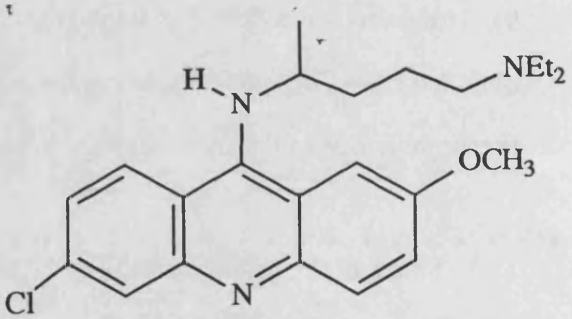
28 R = H

33 R = N_3



29 R = H

32 R = N_3



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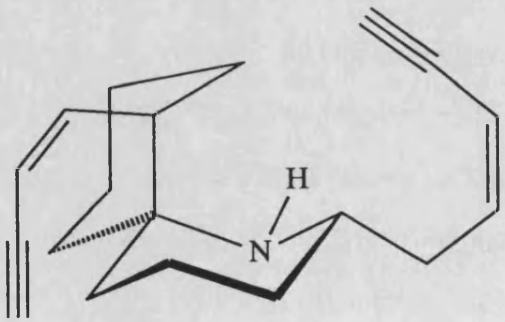

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It has been shown[28] recently that it is possible to photoaffinity label the nAChR site by use of an aryl diazonium derivative. The photolabel used was 4-(dimethylamino)benzenediazonium tetrafluoroborate **27** (DDF). On photolysis DDF loses nitrogen to generate a very reactive aryl cation which rapidly becomes covalently bonded to the receptor protein. A degree of hydrophobicity associated with the salt increases its affinity for the non-competitive antagonist binding site (*vide infra*) on the receptor protein. However, in the presence of phencyclidene **28** this binding is prevented and the photolabel is specifically introduced at the competitive antagonist site.

In this experiment it was possible to increase the efficiency of the labelling by inducing the photoaffinity labelling via an energy transfer from a tryptophan residue on the receptor protein. Under direct photolabelling conditions the irradiating light had λ_{irr}= 435nm. Under energy transfer conditions the light used had λ_{irr}=290nm. Activation of the photolabel by this method required the proximity of a tryptophan residue so the incidence of photolabelling was increased. The energy difference between the two methods of activation avoided decomposition of DDF in solution.

Since the time of this study it has been shown that competitive antagonists *e.g.* *d*-tubocurarine **5**, flaxedil **12**, and snake α-toxins act at the level of (or close to) the nAChR which causes depolarisation, blocking the effect of agonists by, it is thought, steric hindrance. Another rather heterogeneous group of compounds, including the aminated local anaesthetics *e.g.* trimethisoquin **29**, quinacridine **30**, phencyclidene **28**, *etc.* and the Colombian arrow poison frog toxin, histrionicotoxin **31**, block the response to acetylcholine in a non-competitive manner.[29] *In vitro* studies have shown that these non-competitive antagonists bind to a site distinct from the nAChR that causes depolarisation, but that their binding does have a modulatory effect on the binding of agonists *i.e.* the nAChR is an allosteric protein. Attempts have

therefore been made to distinguish between and photolabel these sites.

A derivative of the non-competitive antagonist trimethisoquin that has been used in photolabeling studies of the electroplaque synapses of *Electophorus electricus* is 5-azido trimethisoquin **32**.[30] This compound can be radiolabelled by quaternisation of the least hindered aliphatic tertiary amine position with [^{3}H]iodomethane. Specific labelling by this compound of the nAChR site of pharmacological action of non-competitive antagonists was indicated by three factors: (1) the labelling was inhibited by histrionicotoxin (another non-competitive antagonist and therefore in competition for the same binding site, assuming there to be only one non-competitive antagonist binding site); (2) labelling was increased in the presence of carbamylcholine **8** (an allosteric interaction); (3)the effect of carbamylcholine was blocked by α-bungarotoxin.

Under these conditions of photoaffinity labelling it was found that two peptide chains were labelled, one with a relative molecular mass of 50,000, the other with a relative molecular mass of 66,000. Three explanations were put forward for the labelling of two rather than one chain: (1) the binding site for non-competitive antagonists was not situated on either chain but they lie in its close vicinity and possess amino acid residues with which the nitrene reacts whilst the non-competitive antagonist is bound to its specific site; (2) the two chains differ from each other but nevertheless possess sites for non-competitive antagonists with similar binding properties; (3) the lighter peptide chain is derived from the heavier one by proteolysis.

These results were later reinvestigated[31] under non-proteolytic conditions and the third hypothesis found to be the correct.

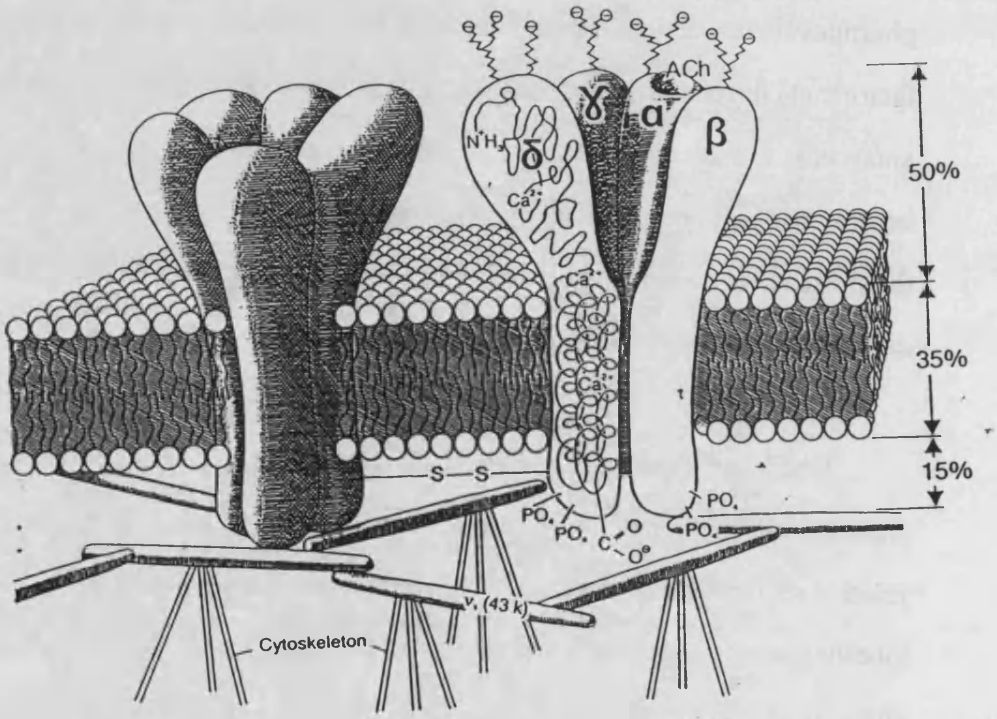


Figure 11 : Schematic representation of the four peptide chains of the nAChR. (Taken from reference 35b).

A derivative of phencyclidene has been used in the photolabelling of the non-competitive antagonist binding site.[32] The ligand used was tritiated 1-(1-(3-azidophenyl)cyclohexyl)piperidine **33**. This was incubated with the intact membranes from the electric organs of *Torpedo Californica* in the dark before being photolysed with long wave ultraviolet radiation. After analysis of the reaction products it was found that the photolabel was specifically incorporated into a region of the receptor protein now known to be close to the non-competitive antagonist binding site. However, these workers using the same photolabel under similar conditions, had previously reported labelling to be non-specific,[33] three peptide sub-units being labelled. The greater specificity recently reported is probably due to refinements in their technique.

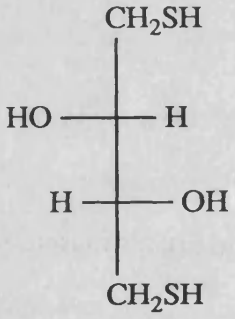
The photolabelling of the non-competitive antagonist site with a derivative of quinacridine **30** has been performed in a continuous flow apparatus.[34] As well as gaining information about the site of photolabelling this experiment also provided information about the mode of ion-gating associated with the nAChR. It was found that it was the peptide sub-unit associated with the nAChR (and the non-competitive agonist binding site) that was mainly photolabelled. The amount of photolabelling of this sub-unit was greatest when the receptor was in its transitory active state (in which the ion channel is open) and less when the receptor was resting or in its desensitized state (in which the channel is closed).

These results, along with others from different techniques, makes the nAChR from fish electric organ and the vertebrate neuromuscular junction the best understood and characterised transmitter receptor (figure 11).[35] The nAChR from these sources is a transmembrane pentameric protein composed of five peptide chains of four types. The resulting structure is referred to as $\alpha_2\beta\gamma\delta$ in adult tissue, the γ sub-unit is not present in denervated and developing tissue and is replaced by a

sub-unit labelled ϵ to give a structure with the stoichiometry $\alpha_2\beta\epsilon\delta$. The amino acid sequences of the α, β, γ, and δ sub-units has been determined by cloning and sequencing the complementary DNA for the respective sub-unit precursors. All four sub-units are highly homologous (average homology 40%), α and β being more homologous to each other than to γ and δ, which in turn are more homologous to each other than to α and β sub-units. All of the four peptide chains are glycosylated and the total relative molecular mass is approximately 292,000. Electron microscopy of the membrane bound receptor show the five sub-units arranged in a rosette and projecting either side of the membrane. The agonist and non-competitive antagonist binding sites are located on the α-sub-units, the two of which are in non-neighbouring, non-equivalent positions.

Due to the wealth of knowledge accumulated about the nAChR at the neuromuscular junction it has led to a number of preconceptions about the corresponding nAChR in the central nervous system (CNS) and brain. This difference is readily shown with snake α-toxins. It is known that α-bungarotoxin binds virtually irreversibly at the neuromuscular nAChR. However, Renshaw cells in the spinal column, which receive a well defined cholinergic nicotinic input, do not bind this toxin.[36] It is known that α-bungarotoxin fails to antagonise nicotinic transmission in both sympathetic and parasympathetic ganglia, the few reports of inhibition can be accounted for on the grounds of contamination of the α-toxin. However, α-bungarotoxin does label with high affinity a small population of sites in vertebrate brain, purification of which has been carried out by affinity chromatography.[37]

The isolation of the brain nAChR is a much more difficult task than in the case of the peripheral nervous system. There is no tissue analogous to fish electric organs which can be used to obtain sizeable quantities of the nAChR. However, it



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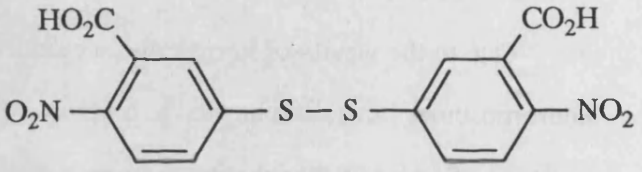

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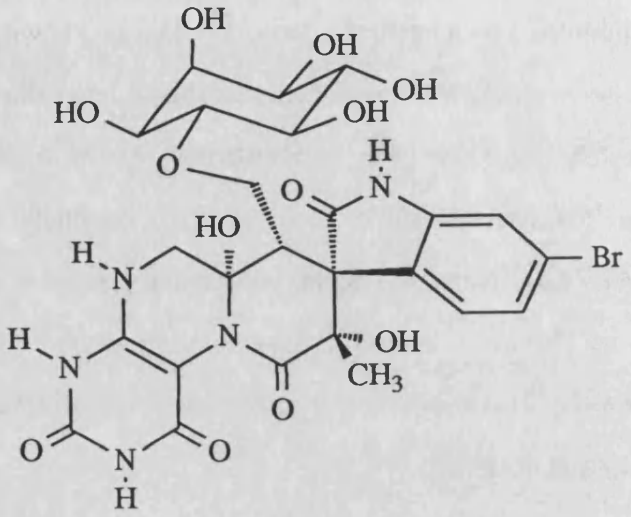
has proved possible to isolate a receptor protein from chick optic lobes using α-bungarotoxin immobilised on an agarose matrix.[37] The receptor was removed from the optic lobe homogenate with this affinity ligand and then desorbed from the matrix with carbamylcholine **8**. From the protein obtained by this method it was found that there were three sub-units present with relative molecular masses of 48,000, 56,000, and 69,000. An amino acid sequencing indicated a high degree of homology between the lightest sub-unit and the α-sub-unit of the nAChR isolated from fish electric organs. This suggests that there may be some relationship between brain and muscle nAChR.

However, other workers[38] have studied the properties of binding sites for radiolabelled acetylcholine, nicotine and α-bungarotoxin. The studies with acetylcholine were performed in the presence of atropine to prevent binding to the muscarinic acetylcholine receptor. Tissue prepared from rat and mouse brains were tested for affinity and density of ligand binding, effects of competitive inhibitors, regional distribution, effects of treatment with dithiothreitol **34** and reversal of these effects with 5,5′-dithiobis-(2-nitrobenzoic acid) **35**, thermal lability, effects of protease treatment and, response to chronic treatment with nicotine *in vivo*. It was found that the binding sites for acetylcholine and nicotine were affected identically for all measurements, whereas the binding site for α-bungarotoxin was affected in a manner different from that for the other two ligands.

Therefore, the problem remains of finding a suitable chemical probe for the nAChR in the CNS and brain. An obvious choice is nicotine, which by definition will bind at the nAChR. Furthermore the psychopharmacology of nicotine suggests that it has a major effect in the CNS.[39] Nicotine tritiated at the methyl position became available in the late 1970's and specific binding of [^{3}H]nicotine to rat brain membranes was first reported in 1980.[40] A consensus of opinion favours two



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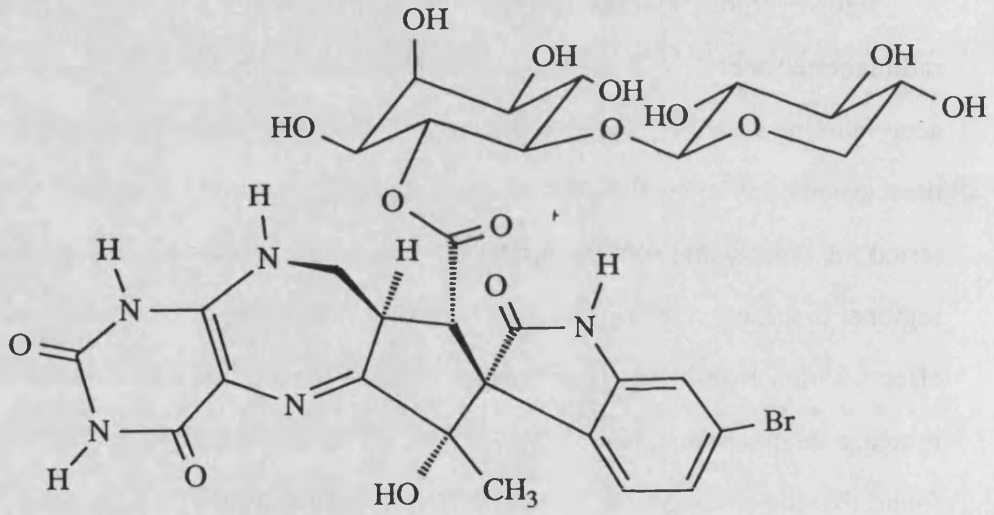

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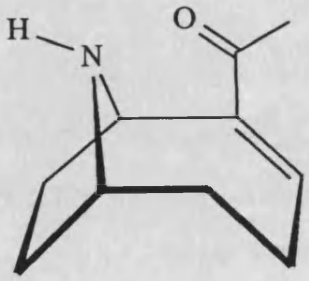

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binding sites, one of high affinity and the other a low affinity binding site which was difficult to characterise because of rapid dissociation of the bound ligand. The low affinity site may correspond to the α-bungarotoxin binding site. However, modification of nicotine for coupling to a solid support for affinity chromatography is difficult to achieve without loss of potency. Clearly, alternative high affinity probes with specificity for this binding site are required.

Nature provides a wide range of structurally and functionally diverse nicotinic neurotoxins. However their use is fraught with difficulties. Of prime importance is the purity of the toxin *e.g.* the potency of surugatoxin **36** (an acetylcholine antagonist which acts at the neuronal nAChR but not at the neuromuscular junction) was found to result from contamination with neosurugatoxin **37**,[41] the true nAChR antagonist in the Japanese ivory shell. Another limitation of naturally occurring toxins, again exemplified by neosurugatoxin, is their scarcity. Therefore, relatively simple structures that can be chemically synthesised have an advantage. This also permits control over their purity and the formation of derivatives. Histrionicotoxin[42] **31** and (+)-anatoxin-a[43-46] (anatoxin) **38** are examples of neurotoxins that have been successfully synthesised.

Anatoxin is produced by the filamentous freshwater blue-green alga *Anabaena flos-aqua* (Lyngb.) de Bréb. Its presence in freshwater algal blooms is responsible for the death of livestock and waterfowl via a depolarising blockade of neuromuscular transmission and subsequent respiratory paralysis.[47] The absolute configuration and stereochemistry of anatoxin have been determined by X-ray analysis of the *N*-acetyl derivative[48] and synthesis from cocaine.[46g] It has been shown by concentration-response studies with isolated muscle tissue to be the most potent of the nicotinic agonists.[49] The similarity in kinetic and steady-state parameters found with muscle tissue for anatoxin and acetylcholine, and the

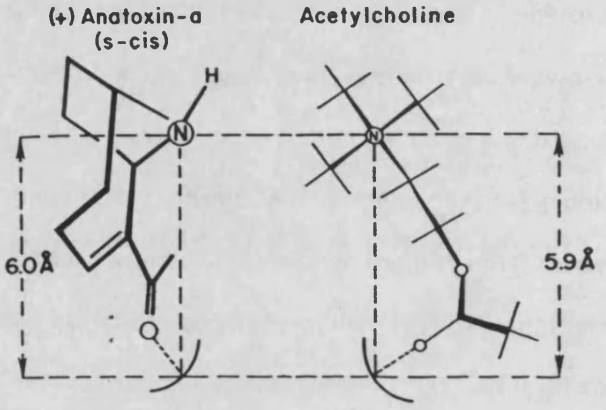


Figure 12 : Structural similarity between (+)-anatoxin-a and acetylcholine (taken from reference 52).

similarity in ionic channel lifetimes indicate that anatoxin does not act by blocking the open ion-channel. As anatoxin contains no ester functionality it is stable in the presence of acetylcholinesterase and will therefore have a longer active lifetime in the synaptic cleft than acetylcholine. Early studies have shown that anatoxin also acts at ganglionic nAChRs in guinea pig ileum[50] and may therefore be expected to be active at the CNS and brain nAChR.

Anatoxin fits geometric models of nicotinic agonists, which suggests that a hydrogen bond forms to a planar region of the agonist and a bulky cationic (charged alkylammonium) group approximately 5.9Å from the hydrogen bond sterically activates the receptor complex (figure 12).[51] Although a secondary amine the toxin is > 99% protonated at the physiological pH of 7.2 (pKa = 9.36).[46a] It has been suggested on the basis of ^{1}H n.m.r. and infrared spectra evidence that the bicyclic structure exists primarily with the seven-membered ring in the twist-chair conformation with the *s-cis* α,β-unsaturated ketone conformation favoured 3:1 over the *s-trans* conformation because of less steric crowding.[46a] The *s-cis* conformation is also suggested to be the preferred agonist conformation because of the optimal distance between the amine nitrogen and carbonyl oxygen.

It has been shown that both (+)-anatoxin-a and (-)-anatoxin-a completely inhibit the specific binding of [^{125}I]α-bungarotoxin but that the (+)-isomer is 50-fold more potent in its activity than the (-)-isomer.[52] In addition the two agonists also stimulate the binding of the noncompetitive antagonist histrionicotoxin **31** to the same degree as they inhibit the binding of [^{125}I]α-bungarotoxin. Anatoxin is therefore a stereospecific nAChR agonist and as such its use in studies of this receptor will allow inferences to be made about the stereochemical requirements of the nAChR. In contrast, it has been shown that the nAChR cannot distinguish between optical antipodes of perhydrohistrionicotoxin.[53]

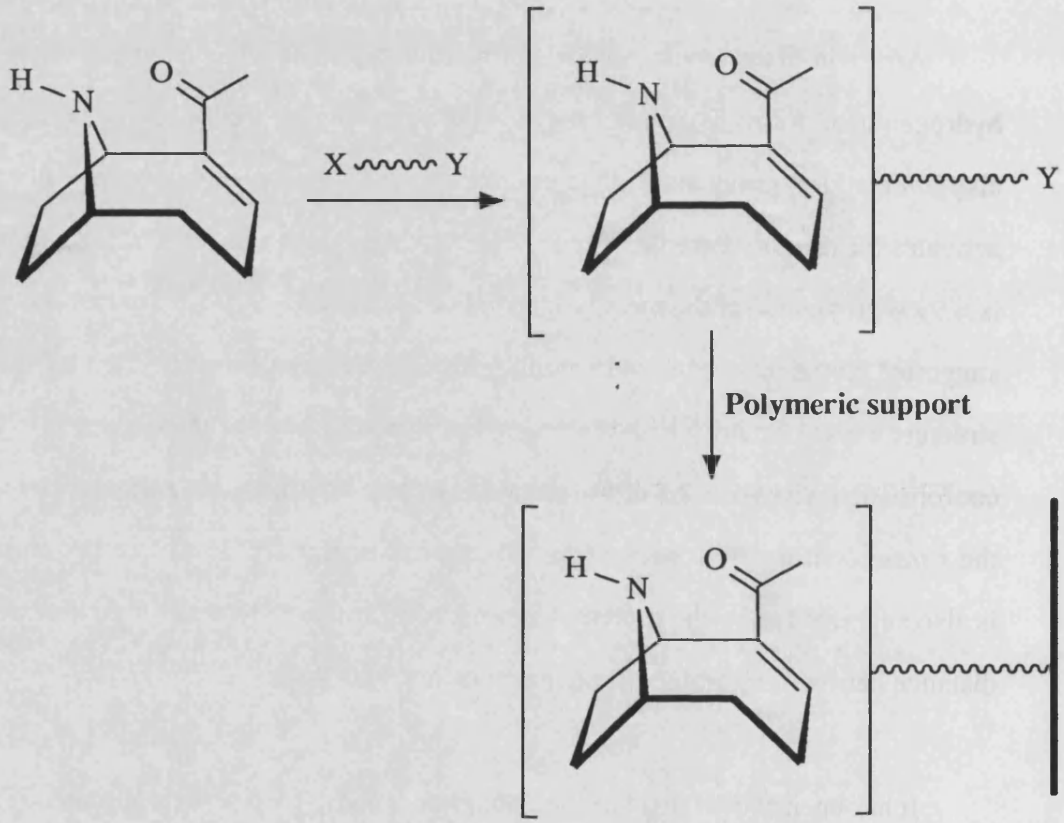


Figure 13 : Anatoxin as an affinity ligand.

Anatoxin should also prove to be a useful probe for the nAChR because of its semi-rigid bicyclic structure which limits the number of accessible conformations compared with an acyclic agonist. In addition to this the α,β-unsaturated ketone group will adopt as near planar a conformation as possible in order to maximise p-orbital overlap, planarity may be prevented due to steric interactions and bond-angle distortion associated with the bicyclic structure. It has been suggested that the *s-cis* conformation is the active conformation (*vide supra*), however, the X-ray structure of *N*-acetyl anatoxin shows a *s-trans* conformation is preferred in the solid phase. The preparation and study of conformationally restricted derivatives of anatoxin should indicate whether it is the *s-cis* or the *s-trans* conformation that is the active form.

If anatoxin is to be used in affinity chromatography it requires the attachment of a functionalised spacer-arm either to anatoxin itself or at some stage during its synthesis (figure 13). Potentially the easiest way to functionalise anatoxin in order to prepare an affinity ligand is by reaction of the secondary amine group with an electrophile. However, it seems logical to presume that both the amine and the α,β-unsaturated ketone are in some way involved in recognition at the receptor site. Derivatisation of anatoxin in such a way that the relative orientation or spacing of these two functional groups is compromised may result in a loss of activity. A suitable synthetic route for the preparation of potential affinity ligands based on anatoxin will allow the introduction of spacer-arms at positions remote from the ketone and secondary amine groups so that they suffer the minimum disturbance.

2. Discussion and results

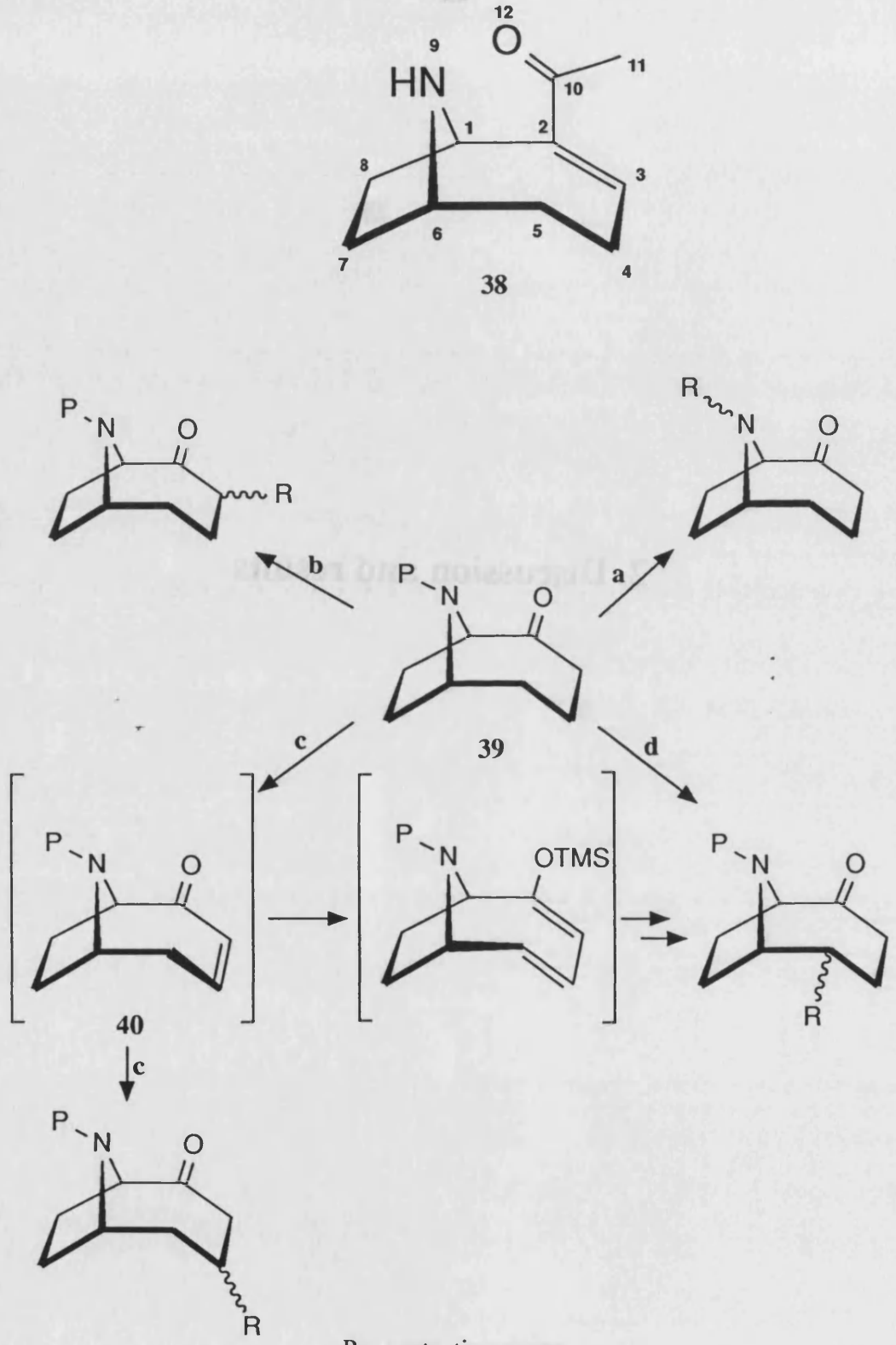


Figure 14 : Possible positions for substituting bicyclic ketone **39**.

2.1. Routes to the 9-azabicyclo[4.2.1]nonan-2-one skeleton.

It was our intention that a probe for the nAChR, based on anatoxin **38**, be synthesised for use in affinity chromatography. This required the attachment of a spacer-arm to anatoxin which contained a terminal functional group so that the derivative of anatoxin could be immobilised by attachment to a polymeric support. Attachment of the spacer-arm to the ligand may require protection of the terminal functionality in the spacer-arm or the use of a suitable synthetic equivalent which could be elaborated without effecting the rest of the molecule. It was important that the point of spacer-arm attachment should have a minimal effect on the binding affinity and specificity of the ligand. A suitable synthetic route should therefore allow attachment of the spacer-arm at several positions of the anatoxin skeleton as no relevant structure activity relationships have been reported for substituted derivatives of anatoxin.

Several published syntheses of anatoxin have involved preparation of the 9-azabicyclo[4.2.1]nonan-2-one skeleton with subsequent homologation of a bicyclic ketone **39** to the α,β-unsaturated methyl ketone group of anatoxin and removal of any protecting groups on nitrogen.[43-45] It was envisaged that bicyclic ketone **39** would allow a degree of latitude in the point of attachment of the spacer-arm (figure 14). Appending the spacer-arm to this bicyclic ketone can be envisaged at: (a) nitrogen, by reaction with a suitable electrophile; (b) α to the carbonyl by reaction of the enolate; (c) β to the carbonyl by conjugate addition to α,β-unsaturated ketone **40**; (d) γ to the carbonyl by addition to a silyl dienol ether. Once formed, the substituted derivative of ketone **39** should be amenable to homologation to a substituted derivative of anatoxin by the existing methodology. Synthesis of anatoxin also makes the C-11 methyl ketone position available for functionalisation via the kinetic enolate.

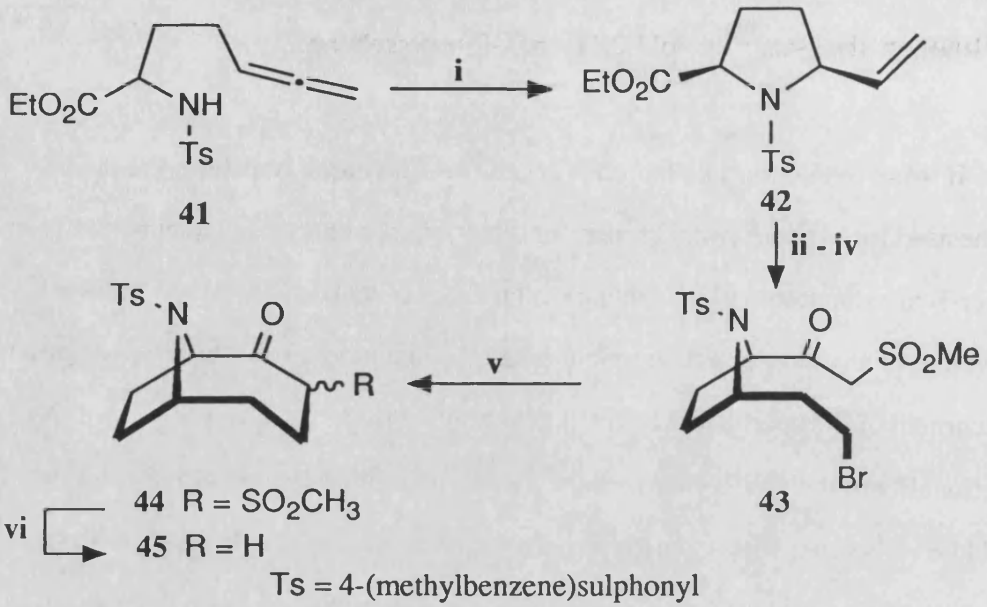


Figure 15 : *Reagents and conditions :* i, $AgBF_4$ (0.1 equiv.), CH_2Cl_2, 24h; ii, B_2H_6, THF, then H_2O_2, NaOH; iii, Me_2SO_2-Bu^nLi (4 equiv.), -10°C, 20min; iv, PPh_3, Br_2, THF, 0°C, 10min; v, NaH, DMSO, 40°C, 2h; vi, Al/Hg, $THF_{(aq)}$, 60°C, 4h .

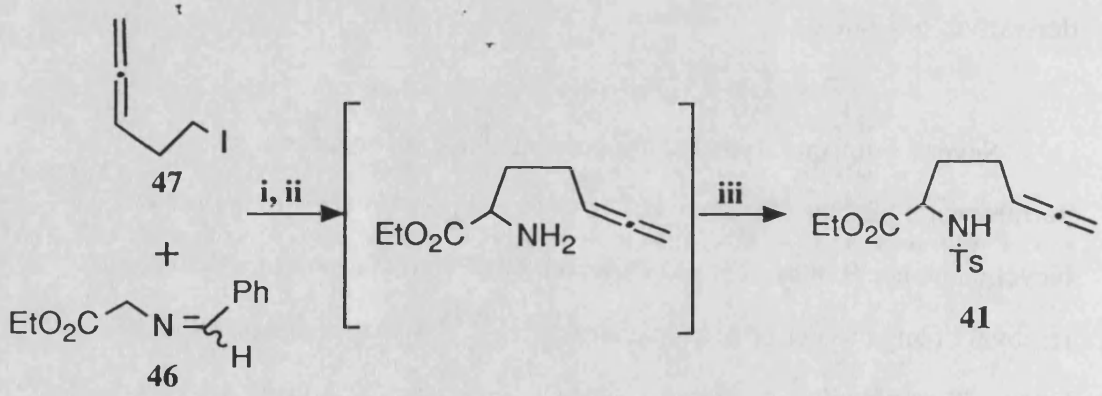


Figure 16 : *Reagents and conditions :* i, **46**, THF, $KOBu^t$, -78°C, 1h then **47**, THF, -78°C to 0°C; ii, 2M $HCl_{(aq)}$, Et_2O, room temp., 1h; iii, TsCl, Pyr, 0°C, 16h.

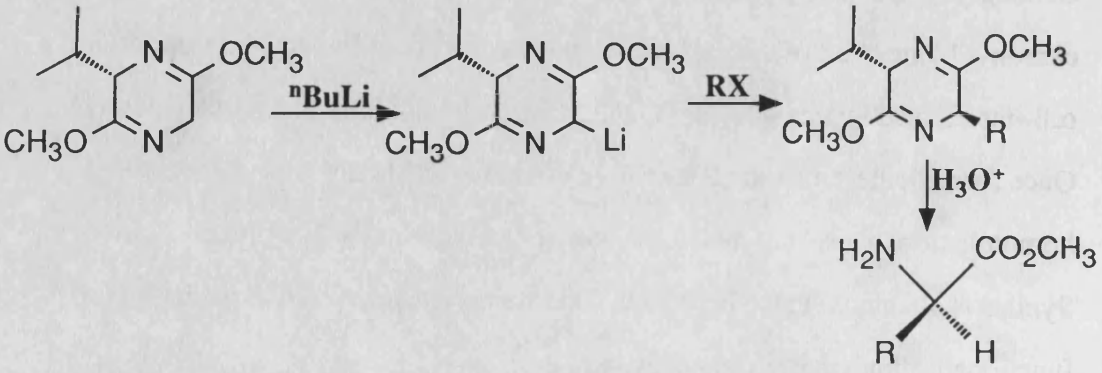


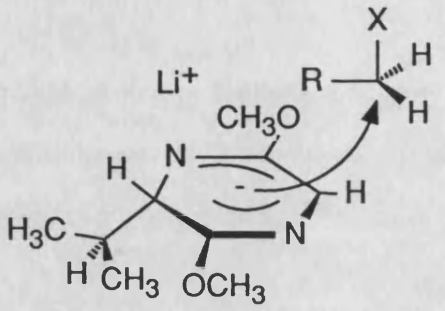
Figure 17 : Schöllkopf chiral glycine anion equivalent.

Due to the requirement to be able to attach spacer-arms at different positions of anatoxin and also because of the known methods for homologating the bicyclic ketone **39** to anatoxin, two synthetic routes towards the 9-azabicyclo[4.2.1]nonan-2-one skeleton were investigated.

2.1.1. Ag(I)-Induced cyclisation of an allenic amino ester.

Previous work within our group has shown that the bicyclic ketone **45** is readily available in racemic form from the allenic amino ester **41** (figure 15).[54] The allene **41** was obtained in 68% yield from the protected glycine ester **46** and allenic iodide **47** in a three step procedure (figure 16). The α-carbanion, formed by reaction of ester **46** with potassium *t*-butoxide, was planar due to delocalisation into the carbonyl and imine π-systems. Therefore no control of the absolute stereochemistry of the alkylated product was possible by this method. The phenyl imine was hydrolysed immediately after alkylation and converted to the sulphonamide **41** before isolation.

We reasoned that racemisation shouldn't occur under the conditions shown in figure 15, and therefore if an efficient route to enantiomerically pure allenic amino ester **41** were available an enantiospecific synthesis of anatoxin should be possible. One method of obtaining the enantiomerically pure ester **41** which has been studied[54] in our group involved the bis-lactim ether methodology of Schöllkopf (figure 17).[55] The chiral induction observed is assumed to arise because the planar dihydropyrazine anion has one diastereotopic face which is shielded by the relatively large *iso*-propyl group. A "folded" conformation **48** is postulated for the transition state, in which the alkyl substituent R is situated above the heterocyclic anion and is thus well positioned to differentiate between the two enantiotopic faces.



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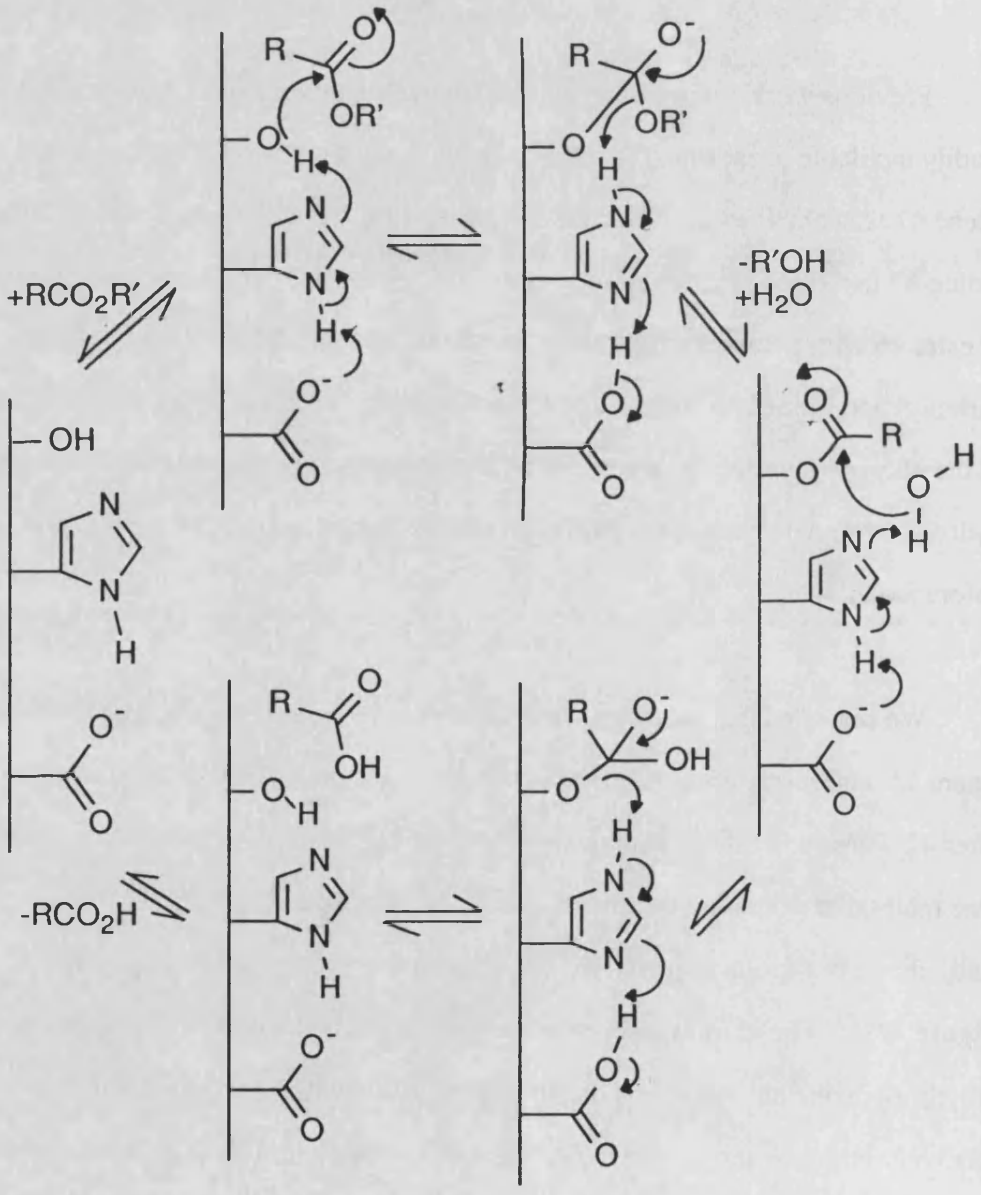
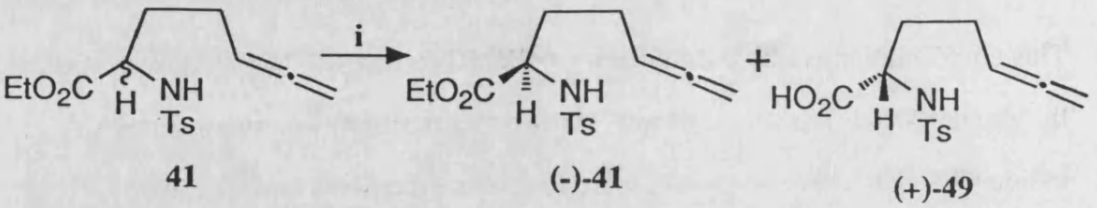


Figure 18 : Ester hydrolysis by α-chymotrypsin.

This conformation is stabilised either by a HOMO (anion)-LUMO (R) interaction or by Van der Waals attraction. However, when this reaction was attempted with iodide **47** an 81% yield of the alkylated bis-lactim ether was obtained which ^1H n.m.r. indicated to be an 8:1 *trans*:*cis* mixture. Attempts to increase the observed enantioselectivity by decreasing the reaction temperature to -100°C resulted in a drop in yield to 13% with a 92%e.e. being realised.

As it appeared that large quantities of ester **41** of high optical purity would be difficult to obtain by this route we turned our attention to resolution of the racemic ester **41.** The use of the inexpensive and commercially available enzyme α-chymotrypsin has been extensively studied for the resolution of amino acids.[56] *In vivo* the enzyme acts as a protein endopeptidase and catalyses, with great specificity, the hydrolysis of non-terminal peptide bonds that are adjacent to phenylalanine, tyrosine or tryptophan residues. An extensive number of *in vitro* studies have been conducted on the ability of α-chymotrypsin to catalyse the hydrolysis of a broad spectrum of amides, esters and other carboxylic acid derivatives.

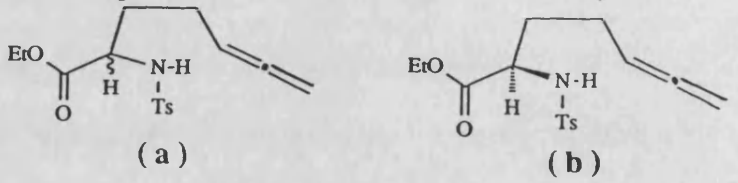
α-Chymotrypsin is thought to hydrolyse esters by the mechanism outlined in figure 18. First the substrate becomes attached to a hydrophobic binding pocket at the active site, usually via an aromatic substituent. An acyl-enzyme adduct is then formed to serine due to negative charge transfer from aspartate through the imidazole group of histidine. The tetrahedral intermediate may be stabilised by hydrogen bonding to a glycine residue in the backbone of the enzyme. The imidazole group of histidine then may act as an acid catalyst to facilitate the liberation of the alcohol, R'OH, leaving behind the acyl-enzyme adduct. The second stage of the reaction is initiated by nucleophilic attack by water. The liberation of the product, RCO_2H, is again assisted by acid catalysis.

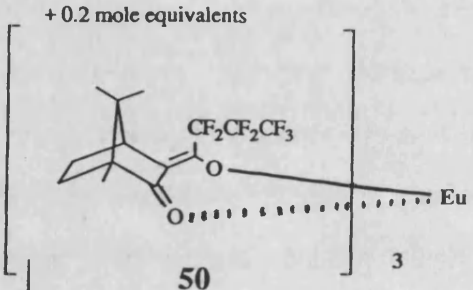


41 **(-)-41** **(+)-49**

$[\alpha]^{20}_{D}$ = 39.8° (c=1.1, $CHCl_3$)

Figure 19 : *Reagents and conditions :* i, α-chymotrypsin (catalytic), acetone/ water 10:1, $NaOH_{(aq)}$ (0.5 equiv.), room temp. .





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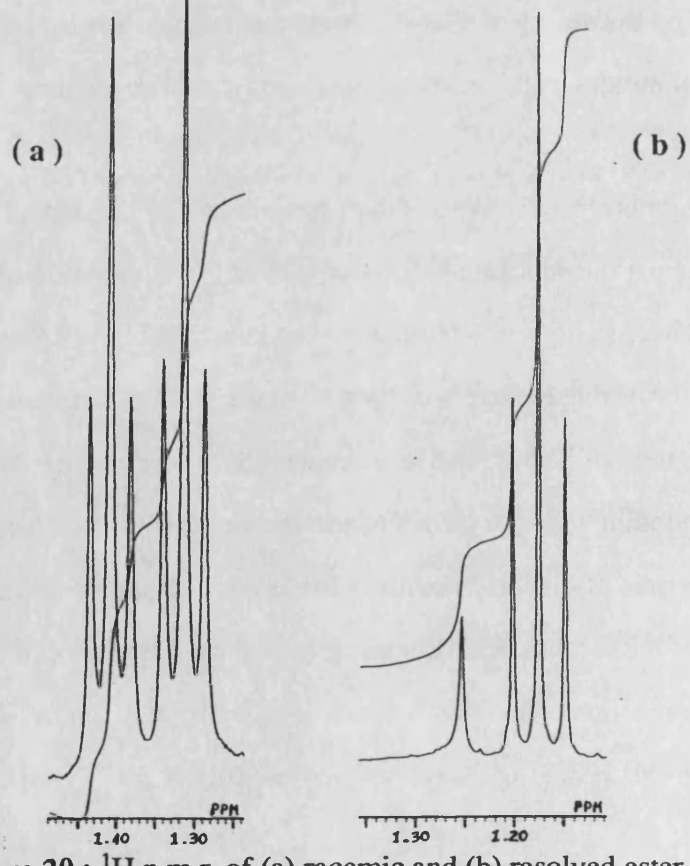


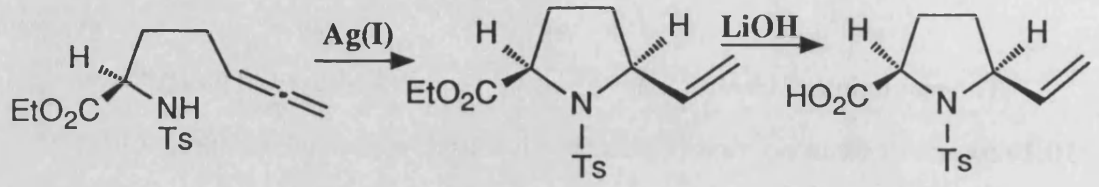
Figure 20 : ^1H n.m.r. of (a) racemic and (b) resolved ester **41** in the presence of 0.2 equivalents $Eu(hfc)_3$ **50**.

α-Chymotrypsin is known to conduct ester hydrolysis in aqueous solutions in the temperature range 0°C-35°C. The optimum reaction rate is found at pH 7.8 and drops off rapidly under more acidic or basic conditions but will still proceed in the range pH 5.8 to pH 9.8. The addition of many organic solvents to aid dissolution of hydrophobic substrates is also possible.

The resolution of racemic ester **41** was performed with α-chymotrypsin in a 10:1 mixture of water:acetone (figure 19). It was essential that a solution of the ester **41** in acetone was precipitated by the addition of water as this caused formation of a fine suspension of the hydrophobic substrate. This ensured free mixing of the enzyme and substrate and resulted in an efficient resolution. The resolution was best performed using an autotitrator to maintain the reaction mixture at pH 7.8 by the addition of aqueous sodium hydroxide. Once 0.5 equivalents of alkali had been added a large decrease in the rate of ester hydrolysis was observed. This was consistent with formation of a pair of diastereomeric enzyme-substrate complexes which exhibited different rates of hydrolysis. After a single recrystallisation ester (-)-**41** and acid (+)-**49** were obtained in high optical purity.

The optical purity of resolved ester (-)-**41** was assessed by chiral shift ^1H n.m.r. (270 MHz) in the presence of 0.2 equivalents $Eu(hfc)_3$ **50**. Under these conditions the racemic ester **41** exhibited two triplets of equal intensity at δ_H 1.31 and 1.41 (J 7Hz, $-OCH_2CH_3$) (figure 20). Under similar conditions the recrystallised ester remaining in the resolution reaction mixture after the addition of 0.5 equivalents of sodium hydroxide exhibited only one triplet at δ_H 1.27 (J 7Hz, $-OCH_2CH_3$). A comparison of intergral heights over this portion of the spectrum indicated the presence of essentially only one isomer.

The optical purity of resolved acid (+)-**49** was also assessed by ^1H n.m.r.



(-)-41

$[\alpha]^{20}_D$= 39.8° (c=1.1, $CHCl_3$)

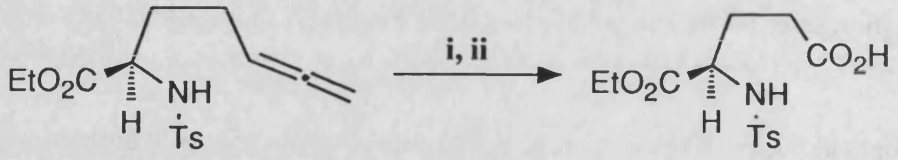
(-)-42

$[\alpha]^{20}_D$= 38.3° (c=2.4, $CHCl_3$)

(+)-51

$[\alpha]^{20}_D$= 9.1° (c=2.2, $CHCl_3$)

Figure 21 : Cyclisation and hydrolysis of resolved ester **(-)-41** .



(-)-41

(-)-52

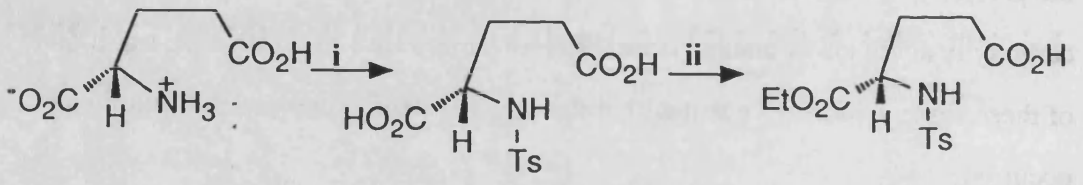
$[\alpha]^{20}_D$= -31.8° (c=1.2, $CHCl_3$)

Figure 22 : *Reagents and conditions :* i, O_3, EtOAc, -78°C;
ii, H_2O_2, reflux, 2.5h.

(270MHz) by formation of a diastereomeric associated ion pair with (R)-α-methylbenzylamine.[54] With racemic acid **49** a splitting of signals at δ_H 5.0 and 7.7 (due to the benzylic and aromatic protons respectively) was observed but the two signals were still overlapping. This made the measurement of optical purity difficult with mixtures of enantiomers. However, the method was used with better success to show that the Ag(I)-induced cyclisation of optically enriched allenic amine (-)-**41** occurred without racemisation at C-2 (figure 21). The racemic pyrrolidine **42** was hydrolysed to the corresponding carboxylic acid with lithium hydroxide. ^{1}H n.m.r. (270MHz) in the presence of 1 equivalent of (R)-α-methylbenzylamine showed splitting of the signals at δ_H 5.0 and 7.7 due to the presence of two diastereomers. However, under similar conditions the carboxylic acid (+)**51** obtained from the resolved ester (-)-**41** exhibited no splitting of these signals, indicating that within the limits of detection no racemisation had occurred.

Before synthesis of anatoxin and functionalised derivatives could be carried out using resolved ester (-)-**41** it was necessary to establish the absolute configuration at C-2. This was performed by derivatisation of allene (-)-**41** and comparison of its optical rotation with a derivative of (S)-glutamic acid. Treatment of allene (-)-**41** with an excess of ozone followed by oxidation of the intermediate ozonide with hydrogen peroxide gave carboxylic acid (-)-**52** in 57% yield (figure 22). An optical rotation of $[\alpha]^{20}_D$= -31.8° (c=1.2, $CHCl_3$) was measured for this compound. No *N*-oxide formation was observed as the sulphone group is strongly electron withdrawing and renders the nitrogen non-nucleophilic and unreactive towards ozone.

The ozonolysis of allenes, it has been suggested, can occur by a variety of mechanisms, depending on the substituents present, giving rise to a range of



(+)-53 **(+)-52**

$[\alpha]^{20}_D$= 21.9° (c=0.9, EtOAc) **$[\alpha]^{20}_D$= 38.5° (c=1.7, $CHCl_3$)**

Figure 23 : *Reagents and conditions :* i, TsCl, 2M $NaOH_{(aq)}$, 70°C, 2h; ii, EtBr (1.1 equiv.), Et_3N (1 equiv.), DMF, room temp., 16h.

products depending on the work-up procedure used.[57] By analogy with the methoxymercuration of allenes studies by Waters and Kiefer,[58] the double bond which suffers initial electrophilic attack depends greatly on the degree of alkylation, the more substituted double bond being favoured. However, in the presence of excess ozone both double bonds will be ozonolysed and the exact reaction pathway(s) will become complicated. Whatever the intermediate formed, it is known that allene ozonolysis products typically fragment to give two carbonyl compounds and carbon monoxide, carbon dioxide not being observed. Presumably under the oxidative conditions of the work-up (hydrogen peroxide) these carbonyl compounds (or their precursors) were oxidised to carboxylic acids, thus affording (-)-**52**.

The optical antipode of carboxylic acid was prepared by the route shown in figure 23. The *N*-tosyl derivative of (S)-glutamic acid (+)-**53** was formed according to the procedure of Harrington and Moggridge in 53% yield.[59] Under the basic conditions of the reaction the zwitterion was converted to the amine dicarboxylate thereby promoting formation of the sulphonamide (+)-**53** with tosyl chloride.

Selective α-ester formation was possible under the conditions of Nefkens and Nivard.[60] The selectivity of the procedure relies on the greater acidity of the α compared with the γ-carboxyl group. The pK_a of the γ-carboxylic acid group (pK_a= 4.25 in glutamic acid) is typical for that of an unsubstituted carboxylic acid (*e.g.* acetic acid pK_a= 4.76) whilst the α-carboxylic acid group (pK_a= 2.19 in glutamic acid) is more than 100 times more acidic. The increased acidity has been ascribed to the electron withdrawing inductive effect of the α-amine which stabilises carboxylate formation.[61] This effect will be increased in the sulphonamide with respect to the amine as the nitrogen is made more electronegative by the electron withdrawing sulphone. Thus, the sulphonamide (+)-**53** was converted to the

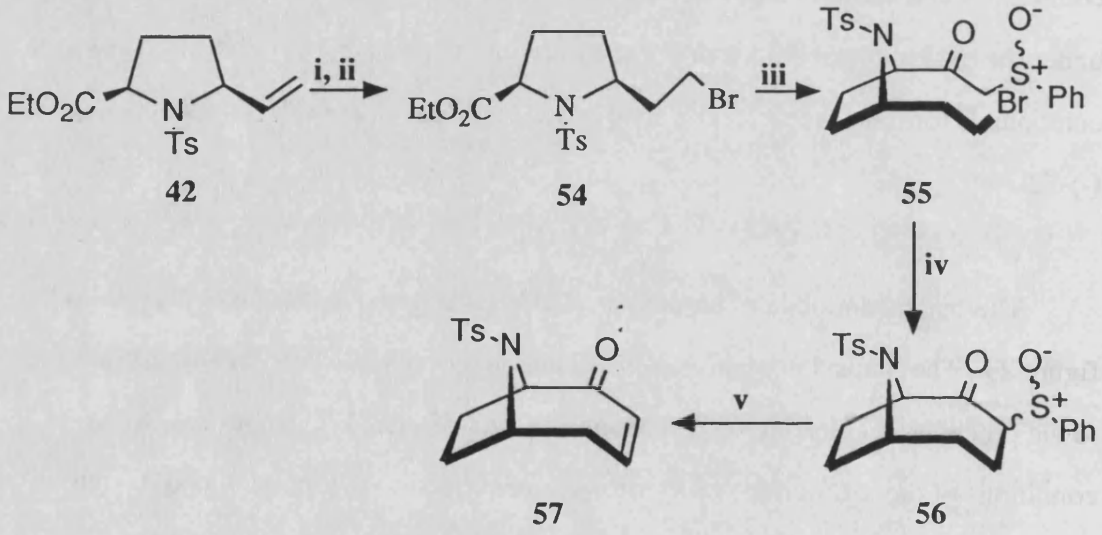


Figure 24 : *Reagents and conditions :* i, B_2H_6, THF, then H_2O_2, NaOH; ii, $PPh_3.Br_2$, THF, 0°C, 10min; iii, MeS(O)Ph, Bu^nLi, -78°C, 20 min; iv, NaH, DMSO, room temp., 2h; v, PhMe, reflux, 3h.

α-amino ester (+)-**52** in the presence of 1 equivalent of triethylamine to selectively form the α-carboxylate. The amino ester (+)-**52** formed by this method had an optical rotation of $[\alpha]^{20}_{D}$= +38.5° (c=1.7, $CHCl_3$), an opposite direction of rotation to that observed for the resolved allene derivative (-)-**52**. The absolute configuration at C-2 of the resolved allene (-)-**41** can therefore be assigned as (R). If the synthetic route shown in figure 15 started with resolved allene (-)-**41** the final product would be anatoxin-a of the same absolute configuration as the naturally occurring compound.

It was interesting to note at this stage that the enzyme displayed a higher preference for the amino acid derivative with the (S)-configuration. This corresponds to an L-amino acid derivative, the configuration of the naturally occurring substrates for α-chymotrypsin. This selectivity occurred despite the presence of the allenic side-chain and the tosyl substituent on nitrogen. However, the enzyme does show specificity for hydrolysis adjacent to amino-acid residues with differing aromatic substituents, so some degree of structural variation is evidently tolerated.

This work therefore made available the ketone **45** with the correct absolute configuration for synthesis of anatoxin and related derivatives. Esterification without racemisation of the carboxylic acid (+)-**49** (obtained by enzymatic resolution) followed by application of the same synthetic route would afford (-)-anatoxin-a. It was envisaged that by a slight modification of the route shown in figure 15 that a shorter synthesis of anatoxin would be possible and which would provide a potentially valuable intermediate for affinity ligand synthesis (figure 24).

As has been reported the allenic ester **41** was cyclised stereospecifically in the presence of silver tetrafluoroborate to give the *cis*-2,5-disubstituted pyrrolidine **42**.[43]

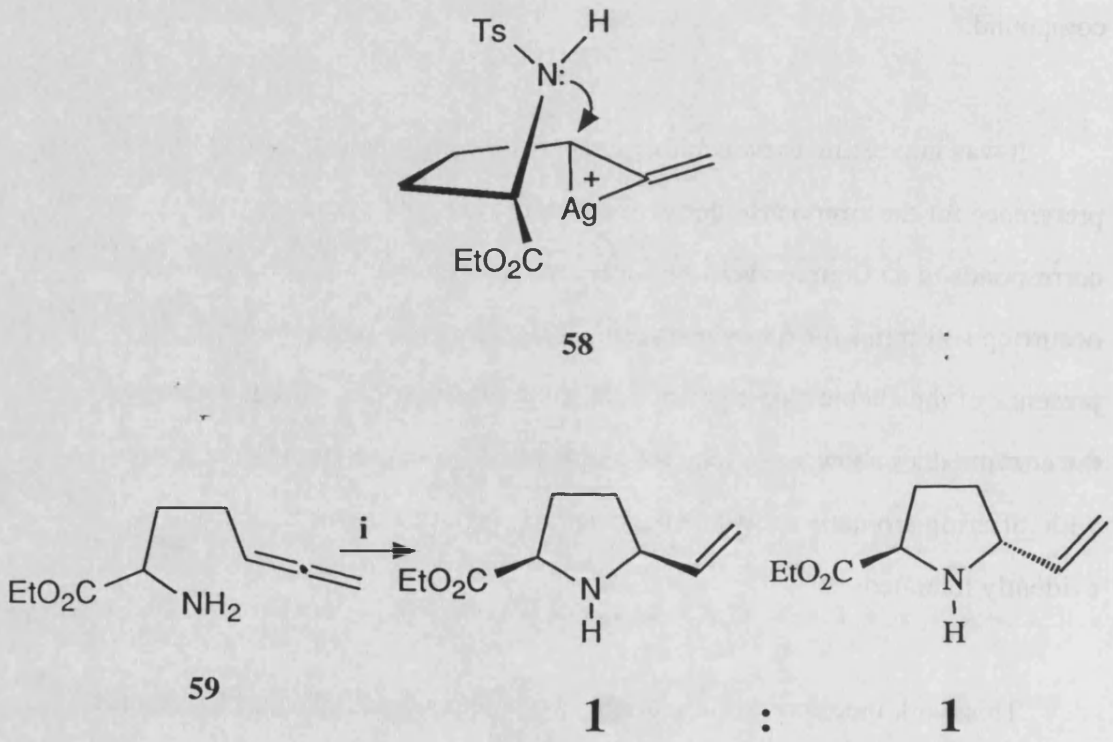


Figure 25 : *Reagents and conditions :* i, $AgBF_4$, CH_2Cl_2, room temp. .

The reaction is considered to proceed via addition of Ag(I) to one of the allene double bonds to form a cationic metallocyclopropane cation which is opened by nucleophilic attack from the sulphonamide nitrogen. The stereospecificity observed in the reaction has been accounted for on the basis of minimisation of steric interactions in a 5-membered "envelope" transition state **58**.

1,2-Interaction between the tosyl and ester groups is minimised by placing the two large groups on opposite faces of the emerging 5-membered ring. The same rationale is used when placing the alkene group on the opposite face to the incoming sulphonamide. The 1,3-interaction between ester and alkene groups is minimised by their positioning at the fold in the envelope structure. This mechanism is supported by the observation that a 1:1 mixture of *cis* and *trans* isomers is obtained when primary amine **59** is cyclised with silver tetrafluoroborate (figure 25). In this case there is no large group on nitrogen to influence the orientation of the alkene sidechain relative to the ester via adjacent 1,2-interactions.

The primary alkene **42** was reduced in a regiospecific manner with freshly produced diborane to give the organoborane arising from anti-Markovnikov addition. Reaction *in situ* of the organoborane with sodium hydroxide / hydrogen peroxide yielded the corresponding primary alcohol in 56% yield. The primary alcohol was cleanly converted to the alkyl bromide **54** with triphenylphosphine dibromide in 87% yield (57% overall from the alkene).

We speculated at this time that an improved yield of bromide **54** may be possible by the direct anti-Markovnikov hydrobromination of primary alkene **42**. Brown and Lane have reported a procedure for effecting this conversion which involves a base induced bromination of the organoborane, which could be formed efficiently as shown by the two step synthesis of bromide **54**.[62] When trialkyl-

(Eqn. 1) $R_3B + 3Br_2 + 4NaOH \longrightarrow 2RBr + ROH + B(OH)_3 + 4NaBr$

boranes are treated with bromine in methanolic sodium hydroxide the corresponding alcohols are formed as major products. The alcohol is thought to arise due to oxidation of the organoborane with sodium hypobromite, formed *in situ* by reaction of hydroxide with bromine. By changing to a simultaneous dropwise addition of bromine and sodium hydroxide, the yield of primary bromides can be increased to approximately 67%, the maximum theoretical yield allowed by the stoichiometry of the reaction (equation 1).

The undesirable oxidative side reaction which gives rise to the alcohol can be circumvented by using sodium methoxide in methanol as base, in place of sodium hydroxide. Presumably the reaction of bromine with sodium methoxide does not result in formation of sodium hypobromite. By this method near quantitative yields of primary alkyl bromides from terminal alkenes have been reported.[62]

When this procedure was applied to terminal alkene **42** a plethora of components were observed by t.l.c.. Isolation and identification of these components was not attempted. Due to the reasonable overall yield of the two step procedure (57%) and its amenability to use on a large scale (≥ 10g) further refinement of these reactions was not of urgent concern.

The ester **54** reacted with lithiomethyl phenyl sulphoxide to give a 76% yield of β-ketosulphoxide **55** (figure 24). β-Ketosulphoxide **55** was obtained as a mixture of diastereomers as the sulphoxide sulphur was chiral and racemic. No attempt was made to separate these compounds as the desired α,β-unsaturated ketone **57** could arise by elimination from both diastereomers. A second equivalent of lithiomethyl phenyl sulphoxide was necessary to effect complete conversion to β-ketosulphoxide **55**. Once formed β-ketosulphoxide **55** was immediately deprotonated by a second equivalent of lithiomethyl phenyl sulphoxide as the methylene protons adjacent to

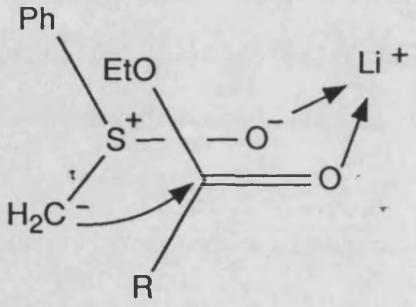


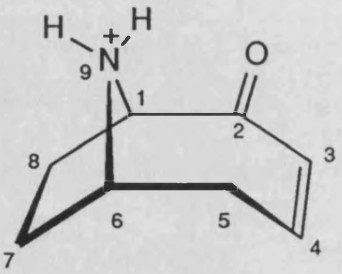
Figure 26 : Possible chelated intermediate to account for chemoselective attack at ester carbonyl.

sulphoxide and carbonyl were more acidic than the methyl protons of methyl phenyl sulphoxide.

β-Ketosulphoxide **55** was obtained as a racemic pair of diastereomers as no attempt had been made to control the absolute configuration of pyrrolidine **54** or the methyl phenyl sulphoxide. Using the method of Kagan methyl phenyl sulphoxide can be obtained in enantiomeric excess by asymmetric oxidation of thioanisole.[63] Reaction of this optically enriched sulphoxide with ester **54** would give a pair of optically enriched diastereomers which may allow for a resolution to be effected at this stage.

In this reaction the methyl phenyl sulphoxide displayed a complete chemoselectivity for displacement of ethoxide by attack at the ester carbonyl, no displacement of bromide was observed. This may be accounted for by assuming coordination of sulphoxide and carbonyl oxygen atoms with lithium cations in solution (figure 26). Coordination of this type would aid attack of the carbanion at the carbonyl carbon via a six-membered transition state (analogous to the proposed Zimmerman-Traxler transition state for aldol reactions).[64] Once displacement of ethoxide has occurred lithium cations can be chelated in a six-membered ring by the two oxygen atoms of the β-ketosulphoxide.

An attempt to carry out the intramolecular displacement of bromide with the lithium enolate of β-ketosulphoxide **55**, formed during the reaction with lithiomethyl phenyl sulphoxide, failed. A similar observation has been made with the corresponding β-ketosulphone **43**.[54] However, when sodium was used as the counter-ion cyclisation could be effected. Thus, when β-ketosulphoxide **55** was stirred with sodium hydride in DMSO one of the methylene protons flanked by the carbonyl and sulphoxide groups was abstracted and the resulting sodium enolate



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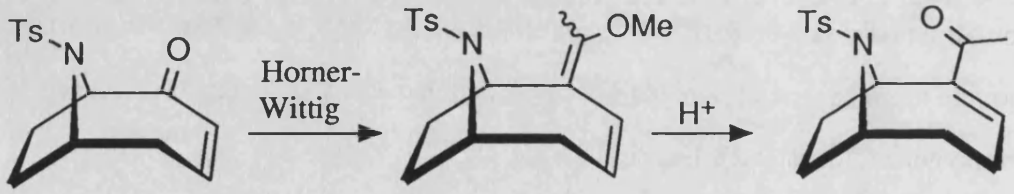


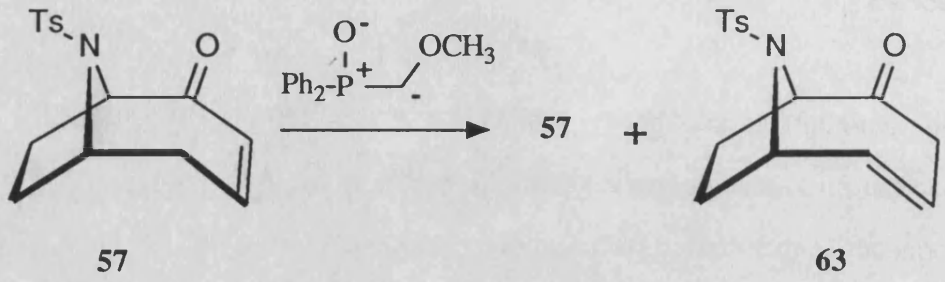
57 **61** **62**

Figure 27 : Proposed method of homologating α,β-unsaturated ketone **57**.

cyclised with displacement of bromide to give the bicyclic β-ketosulphoxide **56** as a mixture of diastereomers in 44% yield. Separation of the diastereomers was not attempted. No product due to cyclisation through oxygen was isolated for this enolate. Cyclisation through oxygen has been observed with the corresponding β-ketosulphone **43**.[54]

Pyrolytic *syn*-elimination of phenyl sulphenic acid was achieved in refluxing toluene to give α,β-unsaturated bicyclic ketone **57** in 48% yield. A carbonyl stretching frequency in the infrared spectrum of this compound was observed at 1665 cm^{-1}. This indicated a high degree of orbital overlap and by inference planarity in the conjugated system. If planar the conjugated system required the presence of three sp^2 hybridised carbon atoms within the [4.2.1]bicycle which would give rise to a certain amount of ring strain for this compound.

To investigate if α,β-unsaturated ketone **57** was unduly strained or deformed from normal bond angles and lengths a brief molecular modelling energy minimisation study on the unsubstituted α,β-unsaturated ketone **60** was performed. These calculations were carried out using the PC Model package which carries out MMX calculations based on the MM2 (QCPE-395, 1977) force field.[65] These calculations indicated that the α,β-unsaturated ketone was approximately 14° from planarity, with a calculated torsion angle of 165.89°. The carbonyl internal bond angle was calculated to be relatively undeformed at 121.14°. The two alkene internal bond angles were significantly distorted from the normal sp^2 hybridised angle of 120°, the C2-C3-C4 bond angle was calculated to be 128.92° and the C3-C4-C5 bond angle calculated to be 130.29°. Therefore, it would appear that there is a significant amount of bond angle deformation associated with bicyclic α,β-unsaturated ketone **57**, a compromise being arrived at by the energetically unfavourable deformation of bond angles so that the α,β-unsaturated ketone can tend



57 63

Figure 28 : Deconjugation of α,β-unsaturated ketone **59** by attempted Horner-Wittig reaction.

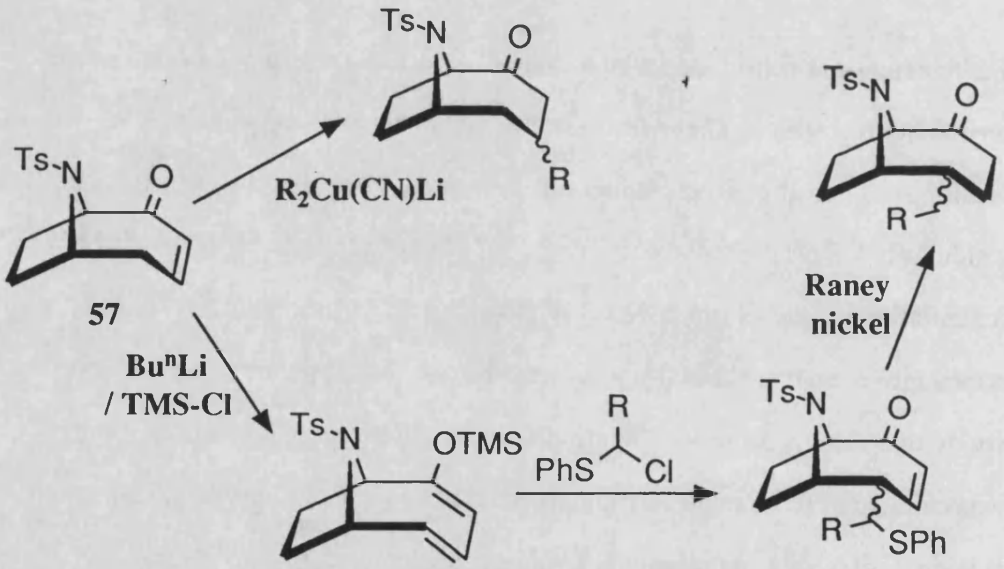


Figure 29 : Possible methods of introducing spacer-arms onto the anatoxin skeleton, for affinity ligand preparation, from α,β-unsaturated ketone **57**.

towards the more stable planar conformation.

We envisaged that homologation of α,β-unsaturated ketone **57** to the α,β-unsaturated methyl ketone group found in anatoxin could be performed in two steps (figure 27). Horner-Wittig olefination of ketone **57** would give vinyl ether **61**, which on hydrolysis of the vinyl ether with acid would liberate the methyl ketone and cause the endocyclic double bond to move into conjugation with the carbonyl group, thus affording *N*-tosyl anatoxin **62**.

It has been shown with ketone **45** that to obtain efficient olefination under Horner-Wittig conditions it is necessary to perform the reaction under argon and using DME as solvent rather than THF.[54] When 1-methoxyethyldiphenyl phosphine oxide was treated with LDA under these conditions an incarnadine solution of the anion was formed. On addition of ketone **57** the colour was immediately discharged, but after work-up none of the expected dienol ether **61** could be isolated.

The reaction was repeated with the anion of methoxymethyldiphenyl phosphine oxide. Addition of ketone **57** to a solution of this anion under the same conditions as before gave a 52% yield of unreacted ketone **57** and 30% of the β,γ-unsaturated regioisomer **63** (figure 28). This result implies that abstraction of a γ-proton is competitive with nucleophilic attack on the carbonyl group, the phosphine oxide anion being strongly basic. The unconjugated ketone **63** arises due to a kinetic protonation of the dienolate α to the carbonyl group.

Despite our failure to convert α,β-unsaturated ketone **57** via Horner-Wittig reaction to anatoxin this compound is still of synthetic interest. Conjugate addition to the α,β-unsaturated ketone or to the derived silyl dienol ether, by the methodology of Fleming,[66] should allow introduction of functionalised spacer-arms

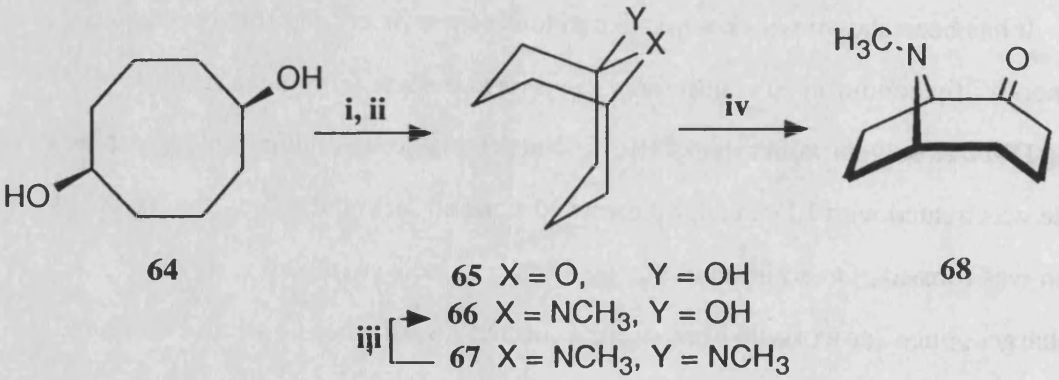


Figure 30 : *Reagents and conditions :* i, PCC, CH_2Cl_2, reflux, 1h; ii, 40% w/v $MeNH_{2(aq)}$, *p*-TSA, reflux, 8h; iii, 10% $H_2SO_{4(aq)}$, room temp., 16h; iv, $C_5H_5N.HBr.Br_2$, CH_3CO_2H, reflux, 16h.

at the C-4 and C-5 positions of anatoxin (figure 29). The resulting ketone should be able to be homologated to an anatoxin derivative using the existing methodology developed for the synthesis of anatoxin from ketone **45**. This work is currently being investigated by a co-worker at Bath.[67]

2.1.2. Oxidative rearrangement of 9-methyl-9-azabicyclo[3.3.1]nonan-1-ol.

Section 2.1.1. outlined a route to bicyclic ketone **45** which could be made enantiospecific. So that methodology for homologation of this ketone and attachment of a functionalised spacer-arm for the preparation of affinity ligands could be studied we required an alternative route to the 9-azabicyclo[4.2.1]nonan-2-one ring system. We required that this route should afford this ring system rapidly and in multigram quantities so that we could undertake further synthetic work with this ketone as an advanced precursor. For preliminary studies a route to the racemic ketone was satisfactory. We employed the oxidative rearrangement route developed by Wiseman and Lee (figure 30).[44]

Jones oxidation of *cis*-1,5-cyclooctanediol **64** has been reported to proceed in 75% yield, but in our hands a yield of 34% was obtained.[68] We found that oxidation was best performed with one equivalent of PCC to give hemi-ketal **65** in 82% yield.[69] No over oxidation to the diketone was observed under these conditions, this is presumably a reflection of the greater stability of the bicyclic hemi-ketal **65** compared to the monocyclic hydroxy ketone isomer. This is supported by the absence of a carbonyl band in the infrared spectrum of hemi-ketal **65**.[68]

The high yield we obtained under these conditions did not reflect the difficulty encountered in the removal of chromium salts. A co-worker at Bath[67] has investigated the possibility of overcoming this problem by carrying out the oxidation

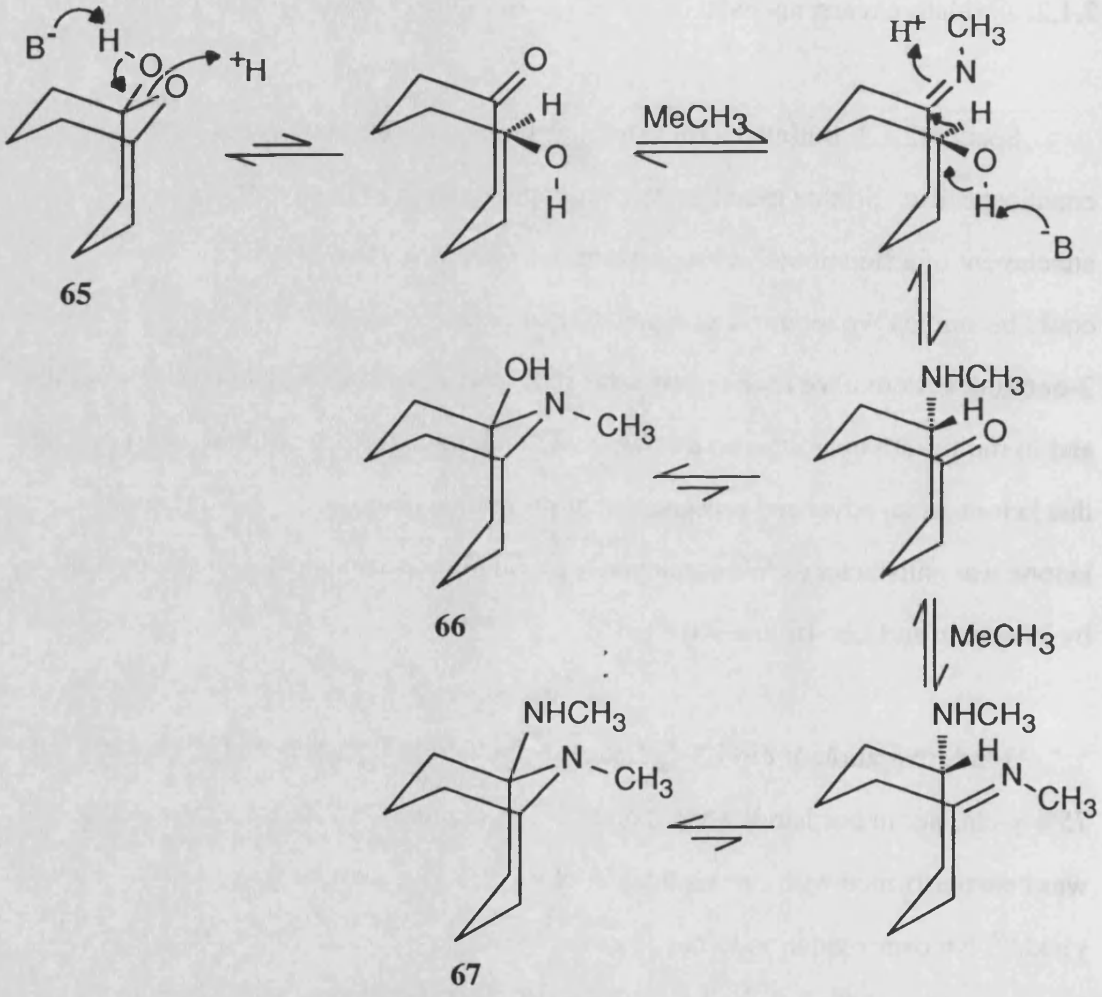


Figure 31 : Formation of aminal **66** and diamine **67** by reversible transannular hydride shift.

with the Jones reagent under phase transfer conditions.[70] Whilst successful, the yield of hemi-ketal **65** fell to 50%.

Treatment of hemi-ketal **65** with methylamine in the presence of an acid catalyst gave hemi-aminal **66** together with diamine **67**. Hemi-aminal **66** was obtained by recrystallisation of the crude reaction mixture while the diamine **67** remained in solution. Diamine **67** was hydrolysed back to hemi-aminal **66** with 10% aqueous sulphuric acid. In this way a 73% yield of hemi-aminal **66** was realised. These transformations evidently involve reversible oxidation-reductions via transannular hydride shifts (figure 31).

Rearrangement of hemi-aminal **66** to the [4.2.1]bicyclo ketone **68** was carried out with pyridinium hydrobromide perbromide. The reaction presumably proceeds by bromination of 5-methylaminocyclooctanone (the monocyclic form of hemi-aminal **66**) and cyclisation with elimination of hydrogen bromide. A yield of 56% for this rearrangement has been reported but in our hands yields were regularly in the 25-27% range despite the purification of all reagents, solvents and the thermostatic control of reaction temperature. However, it was possible to carry out this reaction on sufficiently large a scale (0.5 mol) that sufficient quantities of ketone **68** were available for further work.

We envisaged that this route also offered the opportunity for the preparation of optically active material by using (R)-α-methylbenzylamine in place of methylamine (figure 32). This would allow for separation of optically active diastereomers at a later stage. Hemi-aminal **69** was formed in 21% yield when hemi-ketal **65** and (R)-α-methylbenzylamine were heated in a sealed tube at 100°C for 7 days. Hemi-aminal **69** is a single enantiomer, the only chiral centre being the benzylic carbon atom. The bicyclic structural unit is achiral as a plane of symmetry

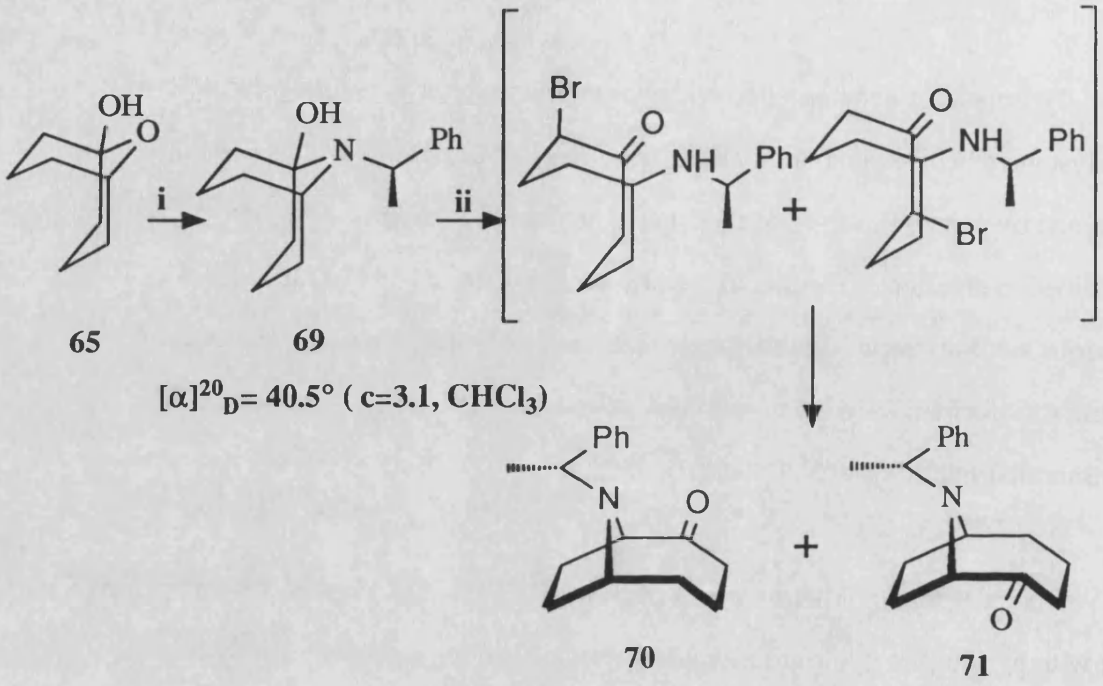


Figure 32 : *Reagents and conditions :* i,(R)-α-methylbenzylamine, *p*-TSA H_2O, sealed tube, 100°C, 7days; ii, $C_5H_5N.HBr.Br_2$, CH_3CO_2H, reflux, 16h.

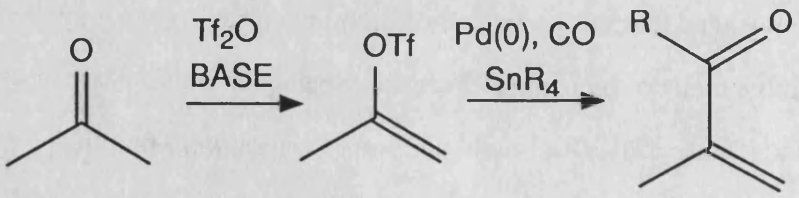


Figure 33 : Vinyl triflate formation and palladium(0) catalysed cross-coupling to organostannane for conjugated ketone formation.

runs through the oxygen, nitrogen, and two bridgehead carbon atoms.

As with the methyl substituted hemi-aminal **66**, rearrangement was performed with pyridinium hydrobromide perbromide in refluxing glacial acetic acid to give a 23% combined yield of **70** and **71**. The two diastereomeric ketones were formed in similar amounts as indicated by ^{1}H n.m.r. (270MHz) but were not readily separable by either analytical or preparative chromatography.

The low overall yield (5%) of ketones **70** and **71** from hemi-ketal **65** meant that while optically enriched compounds were available by this route, significant optimisation of the two steps involved was necessary before synthetically useful quantities of material was available. A suitable method for separation of the two diastereomers was also required. Further studies within this area were not performed.

2.2. Attempted synthesis of anatoxin by carbonylation of a vinyl triflate.

With ketone **45** in hand and with a method available for forming this compound in optically enriched form (section 2.1.1.), we thought that homologation to the α,β-unsaturated methyl ketone may be possible via the vinyl triflate derivative of ketone **45**. Stille and co-workers have shown that vinyl triflates react with carbon monoxide and organostannanes, in the presence of a palladium(0) catalyst, to give the cross-coupled ketone (figure 33).[71] It has been noted that these reactions do not proceed unless 2-3 equivalents of lithium chloride is added to the reaction mixture. It has been suggested for the non-carbonylative palladium(0) catalysed coupling of vinyl triflates with organostannanes that chloride-triflate exchange occurs, to form a

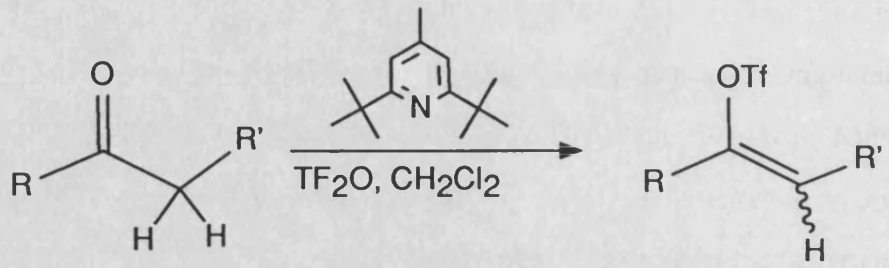
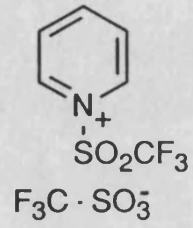


Figure 34 : Use of sterically hindered base in vinyl triflate formation.



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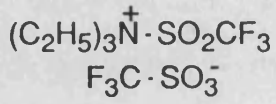


73

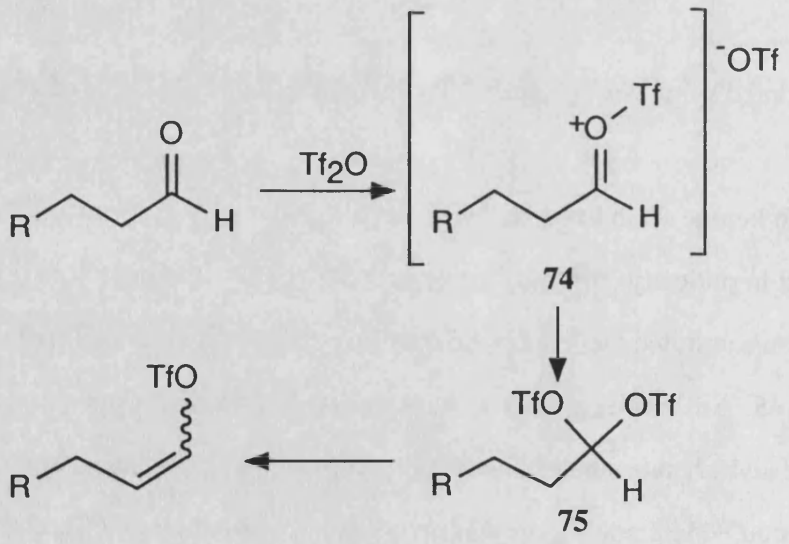


Figure 35 : Mechanism of formation of vinyl triflates derived from aldehydes.

vinylpalladium(II) chloride complex which is then capable of undergoing further reaction.[72] Direct coupling of the vinylpalladium(II) species with the organostannane is competitive with carbon monoxide insertion. At low temperatures carbonylative coupling is slow but at higher temperatures direct coupling predominates. The rate of carbon monoxide insertion can be increased by carrying out the reaction under a higher pressure of carbon monoxide.

The initial synthetic challenge was conversion of ketone **45** to its corresponding vinyl triflate. Several methods exist for effecting this transformation, most of which involve starting from the appropriate carbonyl compound and a source of electrophilic triflate. One of the more recent and general methods available involves the use of the sterically hindered base 2,6-di-*t*-butyl-4-methylpyridine, triflic anhydride and the appropriate carbonyl compound (figure 34).[73] Use of common amine bases, such as triethylamine and pyridine, results in heterogeneous reaction conditions with highly coloured reaction mixtures where the triflating agent is most probably the amine salt **72** or **73**. Due to the steric crowding in the region of the nitrogen atom of 2,6-di-*t*-butyl-4-methylpyridine this base can distinguish between Bronsted (protic) and Lewis acids and so does not react with triflic anhydride to give salts analogous to **72** or **73**. As a consequence the triflating agent under these conditions is the anhydride itself.

Recent studies which used polymer-bound 2,6-di-*t*-butylpyridine have shown that in the case of linear aldehydes exclusive formation of the *gem*-bis-triflate **75** occurs which then thermally decomposes to the vinyl triflate (figure 35).[74] Under similar conditions with cyclohexanone the expected vinyl triflate was formed but no spectroscopic evidence for *gem*-bis-triflate formation was observed. This could be accounted for by *gem*-bis-triflate formation being rate determining and the elimination step becoming relatively fast (figure 36). Hence, there may never exist

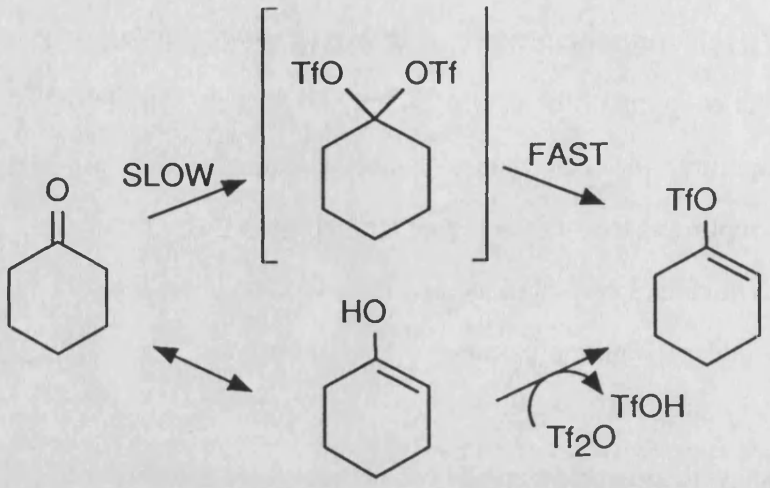


Figure 36 : Vinyl triflate formation from cyclohexanone.

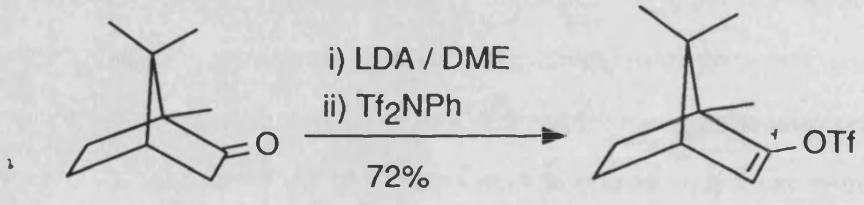
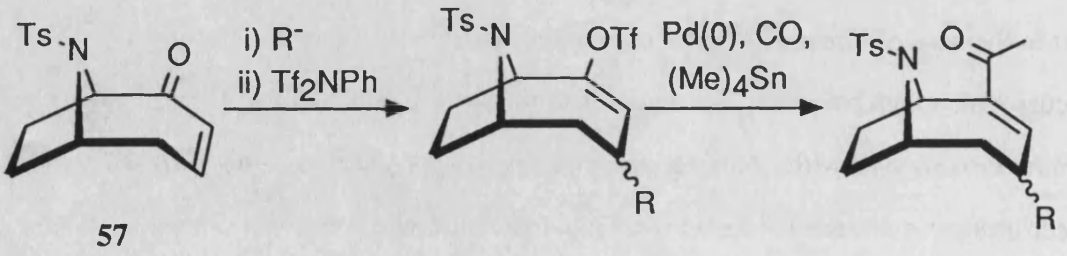


Figure 37 : Vinyl triflate formation from lithium enolate and *N*-phenyl triflamide.



57

Figure 38 : Conjugate addition/trapping with triflate followed by carbonylation to form affinity ligand precursor.

sufficient concentration of the *gem*-bis-triflate for detection. It is also conceivable that the increased steric hindrance associated with a ketone when compared with an aldehyde may preclude formation of a *gem*-bis-triflate and elimination occurs from a cationic species related to **74** (figure 35). These results also fit the previously suggested mode of vinyl triflate formation via reaction of an enol with triflic anhydride (figure 36).[75]

Vinyl triflate formation was attempted using ketone **45**, freshly distilled triflic anhydride in methylene chloride, and 2,6-di-*t*-butyl-4-methylpyridine as base. No consumption of ketone **45** was observed by t.l.c. after 5 days and a strong carbonyl band was still present in the infrared spectrum of the crude reaction mixture. No change in the reaction mixture composition was observed by these techniques when the reaction was heated in a sealed tube at 60°C for 6h. Unchanged ketone **45** was reclaimed in 64% yield.

An alternative method of vinyl triflate formation which has been reported involves trapping the lithium enolate of the carbonyl compound with *N*-phenyl-trifluoromethane sulphonimide.[76] In one case this method was used for the preparation of bicyclic camphor derived vinyl triflate which had previously resisted attempts at preparation, yielding rearrangement products instead (figure 37). Formation of the lithium enolate of ketone **45** was performed with LDA in DME. Attempts to trap the enolate as the triflate were unsuccessful. After 16h a 90% yield of unreacted ketone **45** was reclaimed. This indicated that either enolate formation had been unsuccessful or that trapping of the enolate with the sulphonimide was inefficient.

Despite our inability to either form or trap the lithium enolate of ketone **45** it may prove possible to trap the intermediate enolate formed by the conjugate

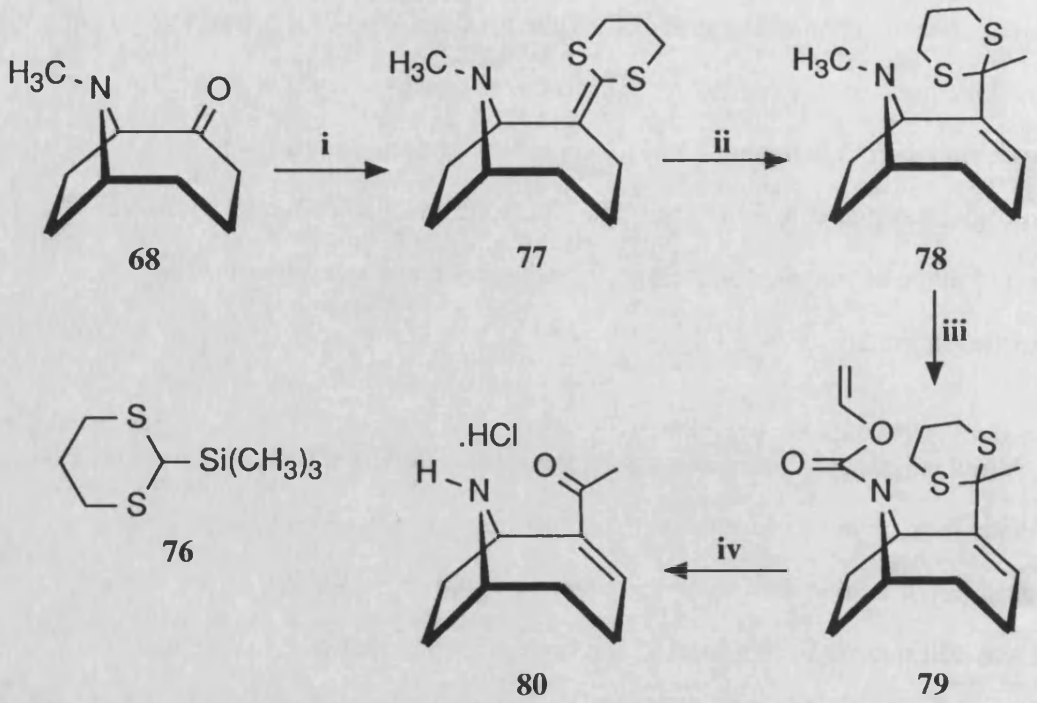


Figure 39 : *Reagents and conditions:* i, **76**, Bu^nLi, THF, -78°C to 0°C; ii, Bu^nLi, THF, HMPA, -78°C to room temp., 3h then CH_3I, -78°C; iii, vinyl chloroformate, CH_2Cl_2, reflux, 16h; iv, 3.6M $HCl_{(aq)}$, *p*-dioxane, DMSO, reflux, 6h.

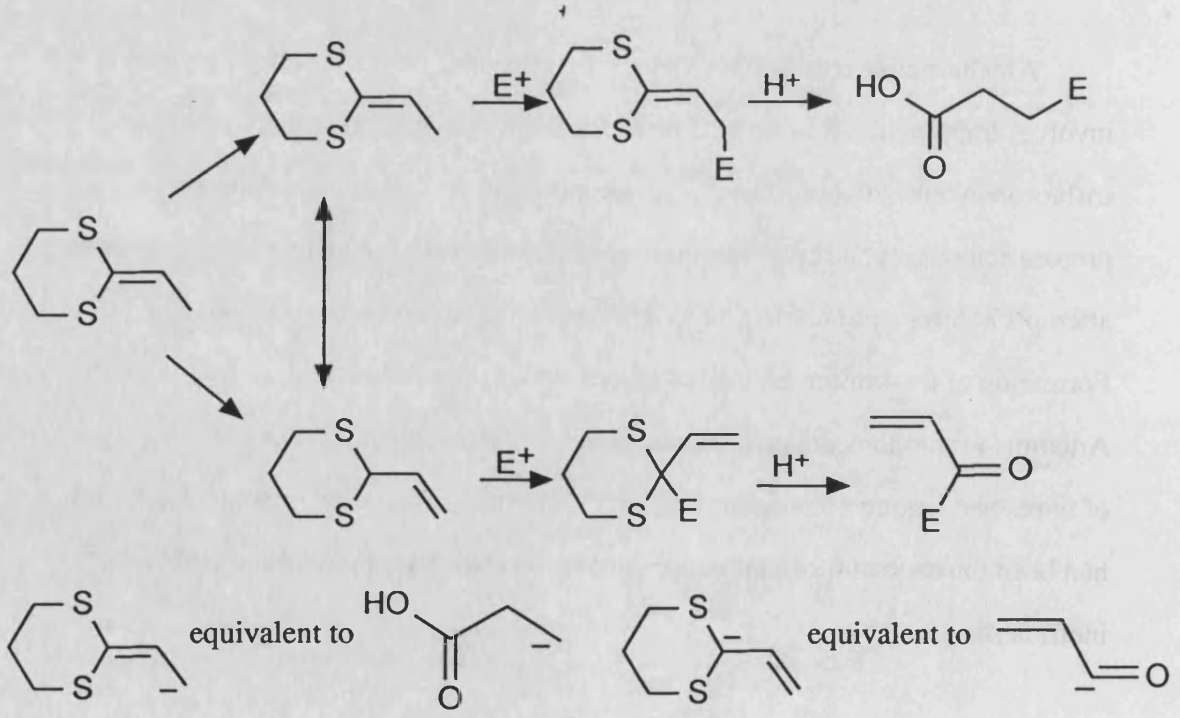


Figure 40 : Dual synthetic equivalence of allylic anion derived from a ketene-*S*,*S*-ketal.

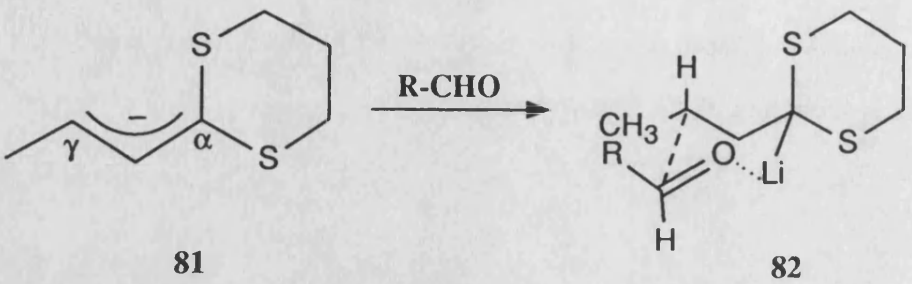
addition to the α,β-unsaturated ketone **57** (figure 38). This would introduce a spacer-arm and allow a rapid preparation of the anatoxin skeleton through palladium catalysed cross-coupling of the resultant vinyl triflate.

2.3. Preparation of the anatoxin skeleton from 9-methyl-9-azabicyclo[4.2.1]nonan-2-one, 68.

As with the tosyl substituted ketone **45** we envisaged ketone **68** being an advanced precursor for the anatoxin skeleton. Two synthetic challenges had to be met in this respect: (a) the bicyclic ketone had to be homologated to an α,β-unsaturated methyl ketone; (b) a successful procedure for *N*-demethylation had to be found. The route developed by Lindgren, Stjernlöf and Trogen (figure 39) for the synthesis of (±)-anatoxin-a appeared to satisfy these requirements.[45]

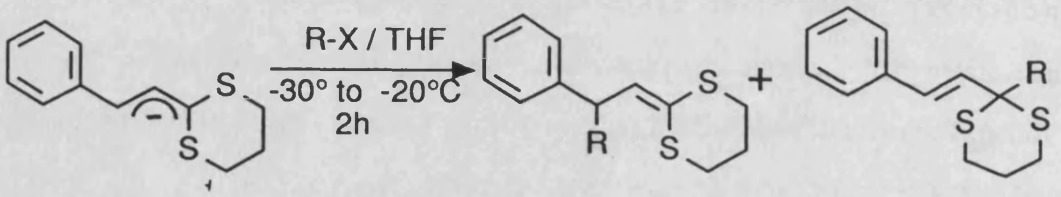
These authors reported trapping of the allylic anion obtained from ketene-*S*,*S*-acetal **77** and n-butyllithium, with iodomethane exclusively at the 2-position of the 1,3-dithiane ring, to introduce the C-11 methyl group of anatoxin. It was our intention that by use of suitable electrophiles in place of iodomethane we could produce affinity ligands based on anatoxin functionalised at the methyl ketone position. For this to be realised it was necessary that electrophilic attack on the allylic anion derived from ketene-*S*,*S*-acetal **77** was regiospecific for the site stabilised by the two adjacent sulphur atoms.

Whilst the anions of 1,3-dithianes are synthetically equivalent to acyl anions their vinylogs, ketene-*S*,*S*-acetal anions, are ambident nucleophiles. They can react with electrophiles either α to the two sulphur substituents of the 1,3-dithiane,



81 82

Figure 41 : Proposed transition state to account for γ-selectivity observed with aldehydes.



83 84 85

R	X	YIELD 84 + 85	RATIO 84 : 85
$C_6H_5CH_2$	I	60	90 10
	Br	75	86 14
	Cl	66	78 22
	OTs	74	74 26
$^nC_3H_7$	I	79	37 63
	Br	57	27 73
CH_3	I	60	44 56
	OTs	80	18 82
	OSO_2CH_3	55	-- 100

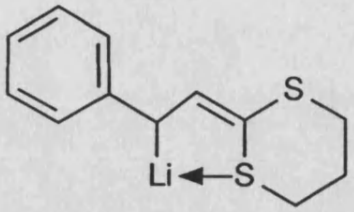
Table 1 : Variation of regiochemistry with electrophile and leaving group.

making them synthetically equivalent to an α,β-unsaturated acyl anion (the desired mode of reactivity), or γ to the two sulphur substituents of the 1,3-dithiane making them synthetically equivalent to a β-propionate anion (figure 40).

Reaction of the crotyllithium species **81** (obtained by proton abstraction from (1-propen-1-yl)-1,3-dithiane with n-butyllithium) with aldehydes has been found to occur regioselectively at the γ-position with high diastereoselection for the *anti*-isomer.[77] This selectivity was accounted for by a proposed chair-like chelated transition state **82** (figure 41). The original *E*-configuration of the alkene was retained in the chair form to avoid interaction of the methyl group with the 1,3-dithiane ring. The R group was favourably orientated equatorial to yield the observed *anti*-configuration of the γ-adducts. The chelated transition state was further supported by the observation that addition of the cation coordinating agent HMPA caused a decrease in γ-selectivity.

The same organolithium species with ketones displayed selectivity for formation of the α-adduct (except with sterically demanding ketones) with little diastereoselectivity. This regiochemical preference cannot be accounted for on steric grounds. Considering the α-position to be more hindered than the γ-position it would be expected that ketones would exhibit an increased γ-selectivity compared with aldehydes.

These results have been rationalised on the basis of the hard and soft acids and bases principle (HSAB principle).[78] The α-carbon of crotyllithium **81** is a relatively hard nucleophilic centre due to polarisation by the two sulphur atoms, as evidenced by the complete selectivity of the very hard electrophiles obtained from D_2O and chlorotrimethylsilane for this centre.[77] The hardness of a carbonyl group can be assessed by the hardness of the substituents on the carbonyl carbon. It is considered



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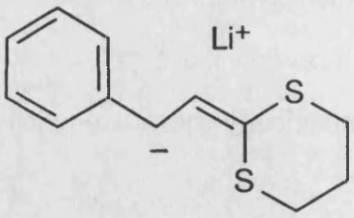

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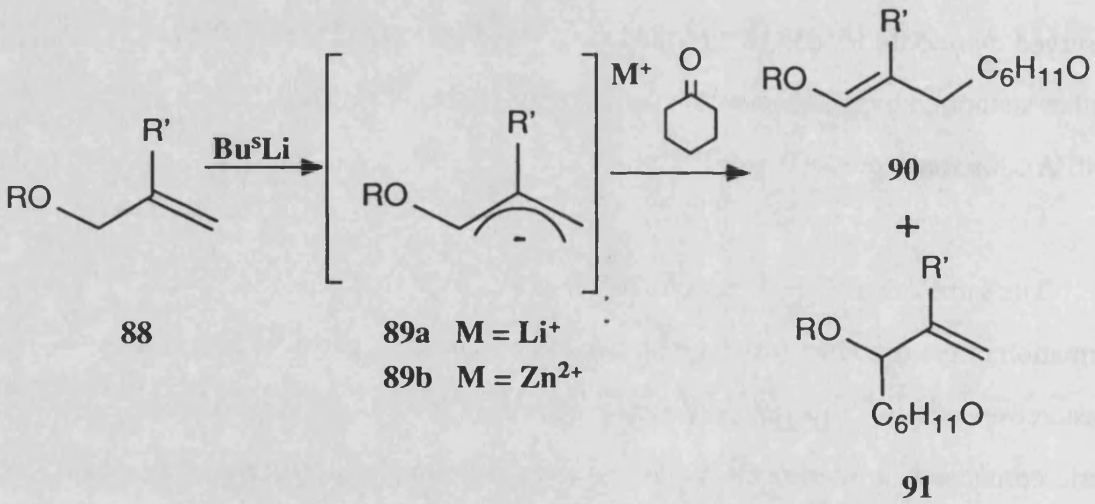


88

89a M = Li^+

89b M = Zn^{2+}

ANION	YIELD / %	RATIO
	90 + 91	90 : 91
89a (R=^{t}Bu, R′=H)	72	27 73
89a (R=Me, R′=H)	72	72 28
89b (R=Me, R′=H)	92	-- 100
89a (R=Et, R′=Me)	93	50 50
89b (R=Et, R′=Me)	97	-- 100

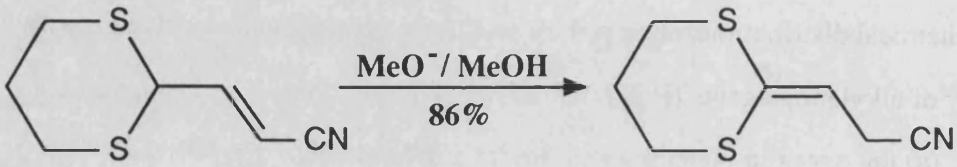
Table 2 : Variation of regio-selectivity with counter-ion and bulk of α-substituent.

that R^- is harder than H^-, so that the carbonyl carbon ($O=C^{2+}/2R^-$) of a ketone (R_2CO) is harder than the corresponding centre ($O=C^{2+}/R^-,H^-$) of an aldehyde (RCHO). For this reason the hard electrophilic centre of ketones added to crotyllithium **81** at the hard α-site while the softer electrophilic centre of aldehydes reacted at the softer γ-site.

The HSAB principle has also been used successfully to explain the regiochemical distributions observed for reaction of cinnamyl derivative **83** with a variety of alkylating agents (R-X) (table 1).[79] The following conclusions can be made : (a) the α:γ ratio increases with increasing hardness of the leaving group ($I < Br < Cl < OTs < OSO_2CH_3$) and the alkyl group ($C_6H_5CH_2 < CH_3 < {}^nC_3H_7$); (b) in the alkylating agent R-X, the influence of X is most dramatic when $R=CH_3$; (c) the product of α-alkylation has the *E*-configuration.

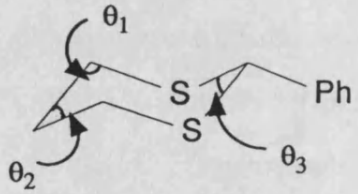
An internally coordinated anion **86** is possible which could explain *E*-alkene formation if planar. Model studies have shown that one isomer should be favoured but do not indicate why that isomer should have the *E*-configuration. However, exclusive γ-metallation would be expected to lower α:γ alkylation ratios, which is not observed. Ziegler and Tam have noted that the regioselectivity of alkylation of ketene thioacetalides was not effected by the addition of HMPA to solvate metal cations.[80] Involvement of the coordinated species **86** is therefore unlikely and the anion **87**, with W-configuration, for steric reasons, is favoured.

Despite the successful use of the HSAB principle for explaining the observed distribution of regioisomers in the reaction of heteroatom stabilised allylic anions, several other factors are also known, or thought, to be important in deciding regioselectivity. It has been shown that increasing the bulk of the oxygen substituent in allylic ethers causes an increase in γ-selectivity for reaction of the



92 93

Figure 42 : Rearrangment of 92 to substituted ketene-*S*,*S*-acetal **93** under equilibrating conditions.



$\theta_1 = 115.5°$

$\theta_2 = 116.5°$

$\theta_3 = 115°$

94

derived allylic anion with an electrophile (table 2).[81] The observation that such substrate steric factors are important in controlling site-reactivity in the reaction of this type of ambident anions with electrophiles is well precedented.[82] The information in table 2 also shows that the nature of the metal counter-ion is also important in determining which site of the allylic anion reacts.

In comparison with the above results it has also been shown that increasing the steric bulk of the electrophilic species when alkylating the anion obtained from allyl silyl ethers increases the amount of γ-alkylation observed.[83]

Another factor which may have some bearing on the regioselectivity of reaction of 1,3-dithiane substituted allylic anions is the ring strain associated with the 1,3-dithiane ring. For example, when 2-(2-cyanoethylidene)-1,3-dithiane **92** was treated with a catalytic amount of sodium methoxide in methanol the only product obtained was the regioisomeric ketene-*S*,*S*-acetal **93** (figure 42).[84]

It may be that there is sufficient 3p-2p π and 3d-2p π overlap of the double bond with the two sulphur atoms to outweigh 2p-2p π overlap in the conjugated nitrile. However, the importance of 3p-2p π overlap in vinyl sulphides has been questioned on the grounds of unfavourable alignment of 3p and 2p orbitals on adjacent atoms.[85] An alternative explanation for this phenomenon is based on the large bond angles found for 2-phenyl-1,3-dithiane **94** by X-ray crystallography.[86]

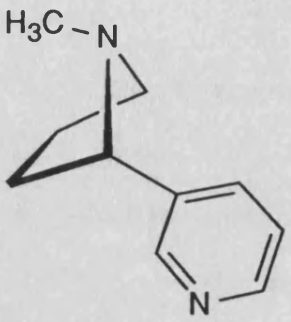
It has been suggested that if the 2-position were allowed to rehybridise from sp^3 to sp^2 this would widen the θ_3 angle to approximately 120° and shorten the S-C bond distance, resulting in a decrease in the already strained θ_2 (116.5°) and the θ_1 (115.5°) angles. There would also be considerable alteration in the dihedral angles between adjacent protons. The rearrangement may therefore be a result of angle

strain relief and added to this the 3p-2p π overlap by the two adjacent sulphur atoms. Also to be taken into account when considering ketene-*S*,*S*-acetal **77** is the difference in strain energy between having the double bond external or internal to the bicyclic system.

We therefore envisaged that by use of suitable metal counter-ions, electrophiles and choice of conditions we would be able to use the allylic anion obtained from ketene-*S*,*S*-acetal **77** selectively as an α,β-unsaturated acyl anion equivalent. Conversion of ketone **68** to the corresponding ketene-*S*,*S*-acetal **77** was achieved by Peterson olefination with 2-lithio-2-trimethylsilyl-1,3-dithiane in 79% yield after purification by chromatography (figure 39). Analytically pure ketene-*S*,*S*-acetal **77** was available by recrystallisation from 60-80 petroleum ether. Recrystallisation from di-*iso*-propyl ether, as previously reported,[45] in our hands was capricious.

With analytically pure ketene-*S*,*S*-acetal **77** in hand alkylation of the derived allylic anion with iodomethane according to the carbonyl-umpolung methodology of Seebach was attempted.[87] Addition of n-butyllithium to a solution of ketene-*S*,*S*-acetal **77** in THF containing HMPA (as reported) resulted in formation of a deep red coloured solution after 3 hours at room temperature. Addition of iodomethane in THF at -78°C gave a 16% yield of 2-methyl-1,3-dithiane **78** and 50% of unreacted ketene-*S*,*S*-acetal **77**, no γ-alkylation was observed. The lowly yield of methylated material compared poorly with the reported yield of 85% for this reaction.

At this stage we decided to synthesise a sample of *N*-methyl anatoxin hydrochloride **95** for binding affinity testing. If *N*-methyl anatoxin retained a high degree of affinity and specificity for the nAChR there would be two important



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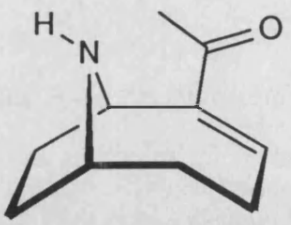

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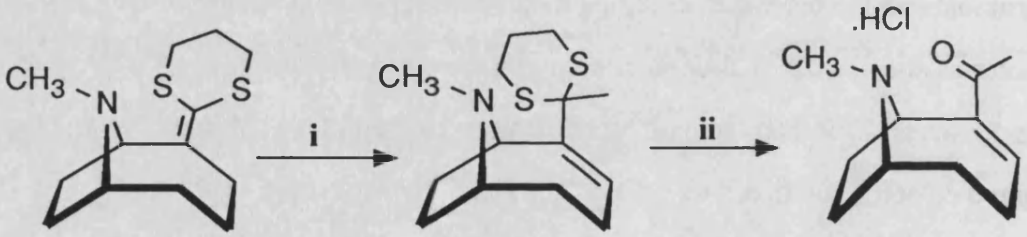


77 78 95

Figure 43 : *Reagents and conditions:* i, Bu^nLi, THF, -78°C to room temp., 3h then MeI, -78°C; ii, 3.6M $HCl_{(aq)}$, *p*-dioxane, reflux, 4h.

implications on future work. Firstly, if the *N*-methylated material satisfied these requirements it implied that an *N*-demethylation step need not be included in the preparation of an affinity ligand, thereby simplifying the synthesis. Secondly, if an alkyl substituent on nitrogen could be accommodated without any deleterious effects on the compounds biological activity it suggested that nitrogen might be a good site to attach a spacer-arm for immobilisation of the molecule.

The biological activity of *N*-methyl anatoxin was also of interest from the point of view that the pyrrolidine nitrogen of nicotine **4** (the compound whose activity anatoxin is mimicking) has a methyl substituent. However, if the pyrrolidine rings of nicotine **4** and anatoxin **38** are superimposed and the pyridine ring of nicotine orientated so that the nitrogen atom is coincident with the oxygen atom of the α,β-unsaturated ketone group of anatoxin it can be seen that the biologically active (+)-form of anatoxin has the opposite absolute stereochemistry to the active form of nicotine.

When hydrolysis of dithiane **78** was carried out with 3.6M aqueous hydrochloric acid in *p*-dioxane containing DMSO (as reported for the anatoxin precursor **79**)[45] low and variable yields of *N*-methyl anatoxin **95** were obtained (22-42%) (figure 43). It has been proposed that in aqueous hydrochloric acid, DMSO is in equilibrium with small (undetectable) amounts of chlorodimethylsulphonium ion, a positive chlorine source which may be used as a mild chlorinating agent.[88] Chlorination of 1,3-dithianes in aqueous media has been used to obtain carbonyl functionality from 1,3-dithianes.

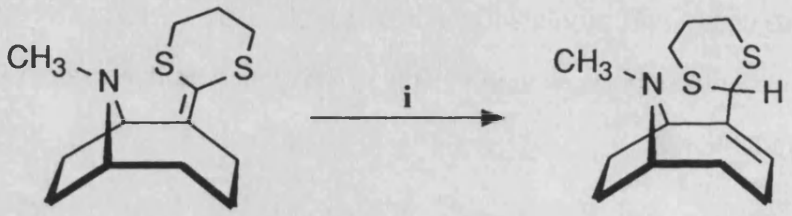
Whilst this may be the case for the rapid hydrolysis of 1,3-dithianes the possibility of an equally effective reaction occurring in the absence of DMSO, the reaction proceeding via protonation rather than chlorination of sulphur, was

investigated. When 2-methyl-1,3-dithiane **78** was heated to reflux in *p*-dioxane containing 3.6M aqueous hydrochloric acid *N*-methyl anatoxin hydrochloride **95** was obtained in 69% yield (figure 43). Complete hydrolysis of substituted 1,3-dithiane **78** took 19h under these conditions whereas hydrolysis had been complete in 3h in the presence of DMSO.

The possibility of forming an affinity ligand based on *N*-methyl anatoxin functionalised at the C-11 methyl ketone position was investigated by use of terminally substituted halo-alkanes as electrophiles in place of iodomethane. We chose as our terminal substituents groups which could be elaborated to carboxylic acids or amines so that the affinity ligand could be immobilised by formation of an amide bond. Polymeric supports containing free carboxyl and amino residues are commercially available.

We initially chose as our terminal substituent a *t*-butyl ester. The *t*-butyl group would be sufficiently bulky to prevent competitive nucleophilic attack at the ester carbonyl and should be readily removable by acid catalysed hydrolysis.[89] However, when the allylic anion obtained from acetal **77** was treated with *t*-butyl 5-bromopentanoate none of the expected α-alkylated product was observed. The electrophilicity of the haloalkane was increased by conversion to the iodide under Finkelstein conditions. Once again reaction was unsuccessful as indicated by ^{1}H n.m.r. of the crude reaction product.

The use of a nitrile as a terminal substituent was also investigated. A nitrile group offered the dual synthetic equivalence of a carboxylic acid (by hydrolysis) and an amine (by reduction). Amide bond formation to immobilise the affinity ligand could therefore be carried out in one of two ways. However, when the allylic anion obtained from acetal **77** was treated with 6-iodohexanitrile all that was



77 96

Figure 44 : *Reagents and conditions :* i, Bu^nLi, THF, -78°C to room temp., 3h then saturated $NH_4Cl_{(aq)}$, -78°C.

isolated was the rearranged ketene-*S*,*S*-acetal **96** with an endocyclic double bond. The identity of this compound was later confirmed by separate synthesis (figure 44). The results of the attempted alkylations with *t*-butyl 5-bromopentanoate and 6-iodohexanitrile suggested that deprotonation of these substrates was occurring instead of nucleophilic displacement of halogen. Both ester and nitrile groups can stabilise an α-carbanion, pK_a= 24.5 and 25 respectively.[90]

To avoid competitive deprotonation of the electrophilic substrate introduction of the carboxylic acid synthetic equivalent was delayed until after the alkylation reaction. Alkylation of the allylic anion was attempted using 1-bromo-4-chlorobutane. We envisaged that the different leaving group aptitudes of the two halogen atoms would prevent formation of a bis-1,4-substituted butane or intramolecular cyclisation onto the nitrogen after the desired alkylation. Conversion of the remaining halogen atom to a carboxylic acid could potentially be achieved by displacement with the anion of diethyl malonate followed by hydrolysis or by conversion to the corresponding Grignard reagent and reaction with carbon dioxide. However, attempted alkylation of the anion of ketene-*S*,*S*-acetal **77** with 1-bromo-4-chlorobutane gave ketene-*S*,*S*-acetal **96** as the only isolable product.

This result prompted us to reinvestigate the reaction with iodomethane as electrophile. When the reaction was repeated under the conditions previously employed a 27% yield of the ketene-*S*,*S*-acetal **96** was obtained. As ketene-*S*,*S*-acetal **96** was isolated it indicated that deprotonation of ketene-*S*,*S*-acetal **77** was occurring. However, alkylation with iodomethane was inefficient under the conditions employed. It is known that organolithium reagents can form aggregates in ethereal solvents such as THF. Formation of these aggregates can have a deleterious effect upon the rate and course of their reactions. The action of additives such as HMPA is generally thought to involve complexation of metal cations,

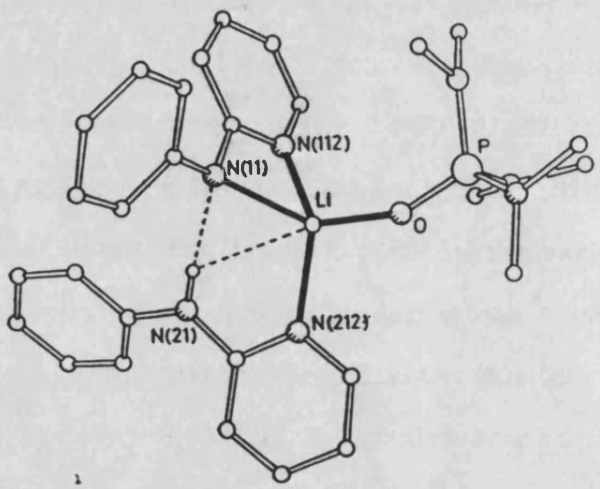


Figure 45 : Crystal structure of [Ph(2-Pyr)NLi].(HMPA).[Ph(2-Pyr)NH]. Structure indicates coordination to both charged and neutral nitrogen atoms by lithium(I). (Taken from reference 92).

causing the decomposition of these aggregates and releasing a more reactive form of the anion.[91]

However, it has been shown that crystalline complexes can be formed between lithium amide bases and HMPA. In addition, X-ray structures of some of these complexes show additional coordination between lithium cations and charged and uncharged nitrogen atoms (figure 45).[92] The possibility therefore arises that while the HMPA is acting to break down organolithium aggregates it is also forming a complex with lithium cations and the tertiary amine. This complex would be over the *exo*-face of the bicycle and may hinder approach of any electrophile until the complex is broken down *e.g.* by the addition of water. The *endo*-face of the allylic anion is less accessible to an electrophile because of the bicyclic framework. The formation of these complexes would be dependant on the relative concentrations of the components and may account for the disparity of the results obtained in our hands and those reported using this set of reaction conditions.

We investigated the reaction of ketene-*S*,*S*-acetal **77** with n-butyllithium *in the absence* of HMPA with view to obtaining a more efficient alkylation procedure and with the added benefit of not having to use highly carcinogenic HMPA. Additives such as HMPA usually show significant rate accelerations for reactions involving organolithium reagents but generally have little effect on the apparent acidities of carbon acids (<2pK_a units).[93] Therefore deprotonation was still expected under these conditions. Addition of 1.2 equivalents of n-butyllithium to a solution of ketene-*S*,*S*-acetal **77** in THF followed by stirring at room temperature for 3h formed a pale yellow solution of the anion. Addition of iodomethane at -78°C gave a 15% yield of reclaimed ketene-*S*,*S*-acetal **77** and 68% of 2-methyl-1,3-dithiane **92** (80% based on reclaimed starting material)(figure 39). Alternatively, quenching of the allylic anion by addition of saturated aqueous ammonium chloride solution at -78°C

gave ketene-*S*,*S*-acetal **96** in 57% yield (figure 44). These results indicate that HMPA decreases the efficiency of alkylation of ketene-*S*,*S*-acetal **77** in the presence of organolithium reagents.

2.4. 9-Azabicyclo[4.2.1]nonan-2-ones with improved chromatographic properties.

The main practical difficulty associated with the use of the *N*-methyl substituted ketene-*S*,*S*-acetal **77** was the poor amenability of this compound to purification and analysis by chromatography on silica gel. Thus, separation of 2-methyl-1,3-dithiane **92** from unreacted ketene-*S*,*S*-acetal **77** required use of a very polar solvent system *e.g.* methanol in diethyl ether or methylene chloride, and components were poorly resolved. The high polarity was ascribed to the basicity of the tertiary amine. Attempts to overcome these problems by chromatography of the hydrochloride salt, addition of triethylamine to the eluant or use of alumina as the stationary phase met with limited success.

We investigated the preparation and reaction of ketene-*S*,*S*-acetals with improved chromatographic performance. We assumed that attachment of an electron-withdrawing group to nitrogen would decrease its basicity and thereby cause a decrease in polarity and improvement in resolution of the compound by chromatography. We also required that these groups be easily removable and stable to the strongly basic conditions of the alkylation reaction. The *t*-butyloxycarbonyl (Boc) and 4-(methylbenzene)sulphonyl (tosyl) groups were evaluated for their suitability in this respect.

2.4.1. Synthesis of *N*-Boc ketene-*S*,*S*-acetal **98**.

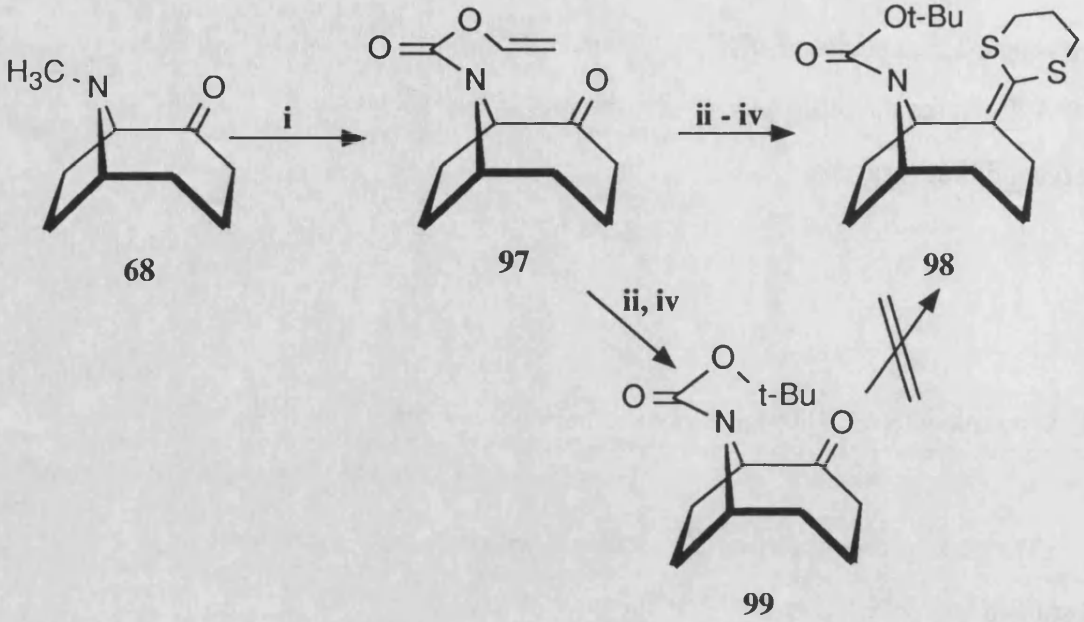


Figure 46 : *Reagents and conditions :* i, vinyl chloroformate, CH_2Cl_2, reflux, 4h; ii, 3.6M $HCl_{(aq)}$, *p*-dioxane, reflux, 7h; iii, **76**, Bu^nLi, THF, -78°C, 1h; iv, $(Boc)_2O$, Et_3N, THF-H_2O (2:1), 16h.

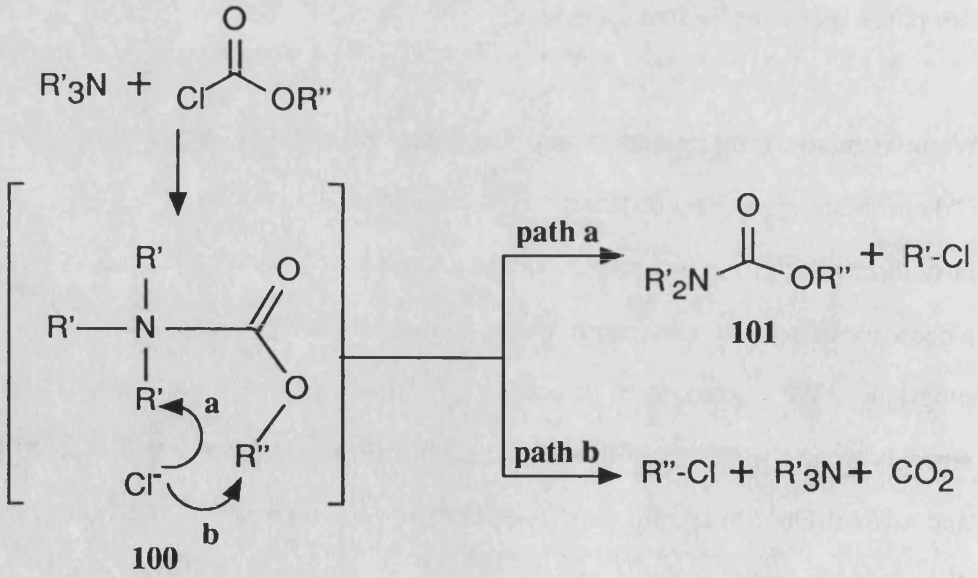


Figure 47 : Mechanism of *N*-dealkylation with chloroformate reagents.

The method used to obtain the *N*-Boc protected ketene-*S*,*S*-acetal **98** is shown in figure 46. The first step in the synthesis of this carbamate was the *N*-demethylation of a tertiary methylamine. Traditionally this conversion has been carried out with cyanogen bromide (the von Braun reaction). The use of cyanogen bromide to carry out this transformation has now been superseded by a range of chloroformate reagents which have proved to be more selective and produce cleaner reaction products.[94] The generally accepted reaction sequence is shown in figure 47. The 1:1 complex **100** formed has two possible fates: (a) nucleophilic attack by chlorine on one of the alkyl substituents on nitrogen (path a) leading to carbamate **101** which can then be hydrolysed to give a secondary amine; or (b) nucleophilic attack of the chloride ion on the *O*-alkyl portion (path b) which has no net effect on the amine.

In 1977 Olofson *et al.* introduced the reagent vinyl chloroformate for use in *N*-dealkylation reactions.[95] The reagent was found to be more selective than any reagent previously used for performing *N*-dealkylations. Only *N*-deethylation, in 90% yield, was observed with vinyl chloroformate and *N*-ethylpiperidine. Previously reported reagents such as 2,2,2-trichloroethyl chloroformate, phenyl chloroformate and cyanogen bromide gave reduced yields or mixtures of products arising from *N*-deethylation and ring scission (figure 48). Olofson attributed the increased yields observed with this reagent to steric factors and the enhanced electrophilicity of the acyl carbon attached to an electron-withdrawing vinyl ether group.

N-Demethylation of the tertiary methylamine **68** was achieved using vinyl chloroformate to give vinyl carbamate **97** in 67% yield (figure 46). Initial attempts to remove the vinyl carbamate by addition of hydrogen chloride to the vinyl group

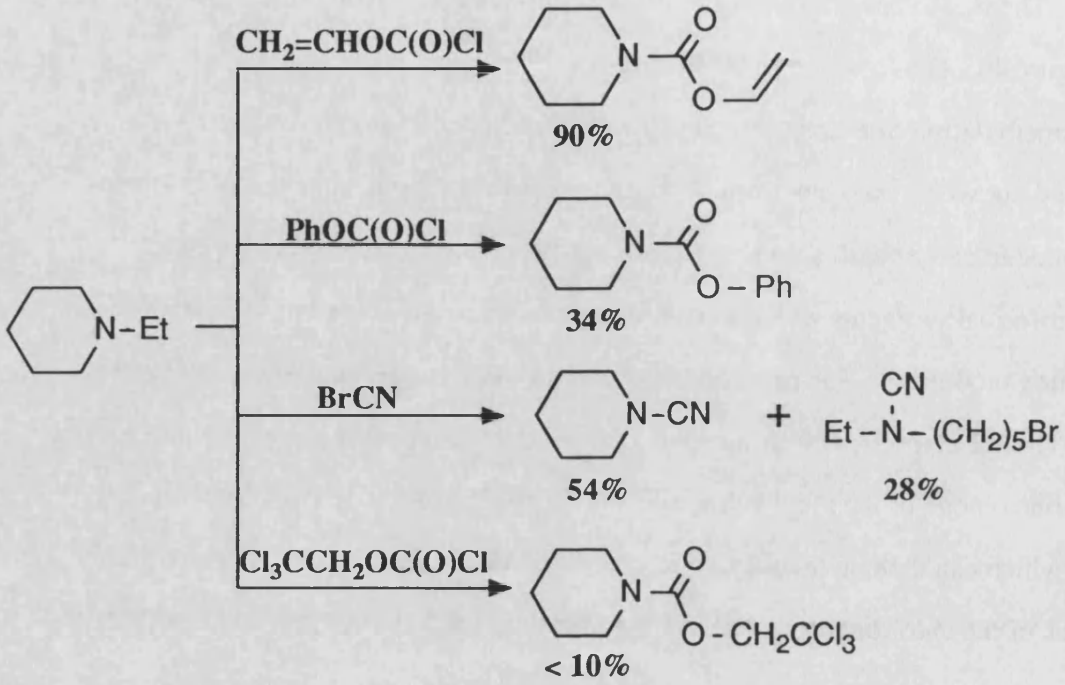
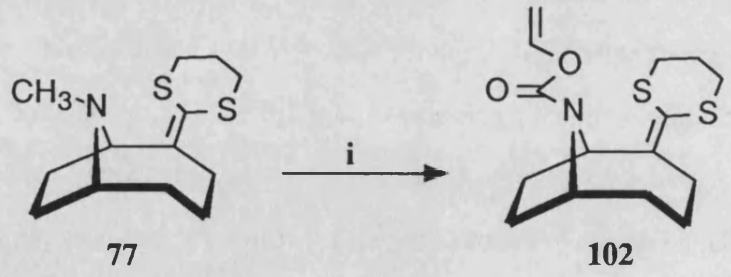


Figure 48 : Efficiency of *N*-deethylation of *N*-ethyl piperidine with various chloroformate reagents and cyanogen bromide.



77 102

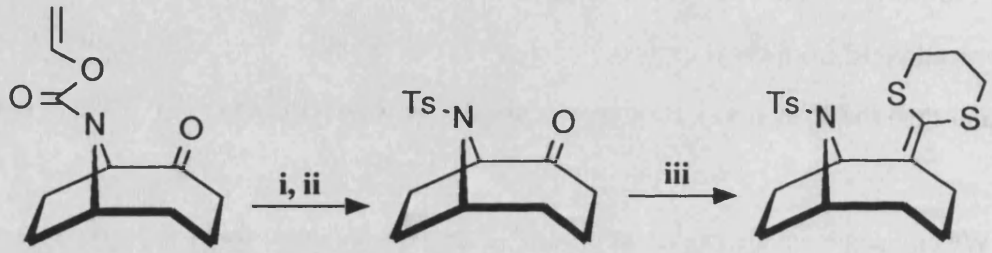
Figure 49 : *Reagents and conditions : i,* vinyl chloroformate, CH_2Cl_2, reflux, 11h.

followed by heating in ethanol to liberate the amine failed.[96] The same deprotection procedure also failed with the vinyl carbamate protected acetal **102** (figure 49), therefore precluding use of **102** as a precursor for *N*-Boc protected acetal **98** as hydrolysis of the ketene-*S*,*S*-acetal would presumably compete with carbamate hydrolysis under acidic conditions.

However, the carbamate **97** was readily hydrolysed to the secondary amine with aqueous acid. Addition of 2-lithio-2-trimethylsilyl-1,3-dithiane to the isolated but unpurified amino ketone resulted in rapid Peterson olefination at -78°C. The ketene-*S*,*S*-acetal formed was protected as its *N*-Boc derivative **98** before isolation in 44% overall yield from vinyl carbamate **97**. As expected this compound was much less polar than the *N*-methyl analogue and was better resolved by t.l.c..

We envisaged an alternative route to *N*-Boc ketene-*S*,*S*-acetal **98** via Peterson olefination of *N*-Boc ketone **99** (figure 46). Acidic hydrolysis of vinyl carbamate **97** released the secondary amine which was directly converted to the *N*-Boc ketone **99** in 67% yield. Attempts to convert ketone **99** to the ketene-*S*,*S*-acetal **98** with 2-lithio-2-trimethylsilyl-1,3-dithiane failed, the infrared spectrum of the crude reaction mixture not exhibiting a carbonyl stretching frequency. It may be that the Peterson reagent is interacting with the carbamate carbonyl group. However, no identifiable product was isolated.

Alkylation of *N*-Boc protected ketene-*S*,*S*-acetal **98** with iodomethane was investigated. Several attempts at this conversion employing the conditions used for the reaction of iodomethane with the *N*-methyl analogue **77** gave reproducible 86-88% yields of unreacted starting material. As allylic anions obtained from ketene-*S*,*S*-acetals are known to react with hard electrophiles, such as protons, at the hard α-position of the allylic anion and no rearrangement was being observed it



97 45 103

Ts = 4-(methylbenzene)sulphonyl

Figure 50 : *Reagents and conditions :* i, 3.6M $HCl_{(aq)}$, *p*-dioxane, reflux, 7h; ii, TsCl, Pyr, room temp., 16h; iii, **76**, Bu^nLi, THF, -78°C, 0.5h.

would appear that deprotonation of ketene-*S*,*S*-acetal **98** was not occurring.[77]

The possibility of forcing allylic anion formation under more vigorous conditions was investigated. Heating ketene-*S*,*S*-acetal **98** with n-butyllithium to 50°C for 6h before quenching with iodomethane gave an 87% yield of starting material. Methyllithium was found to cause extensive decomposition, the only isolable product being unreacted starting material which was isolated in 32% yield. Similarly, deprotonation with *t*-butyllithium was unsuccessful, 64% of ketene-*S*,*S*-acetal being reclaimed.

Our inability to deprotonate ketene-*S*,*S*-acetal **98** may be a result of the steric requirements of the Boc group. The bulky Boc group may render the *exo*-face of the bicycle inaccessible to organolithium reagents, the *exo*-face being relatively inaccessible because of the bicyclic framework. Alternatively the Boc group may be forcing the bicyclic framework into an unreactive conformation, a C-H σ-bond at C-3 not being able to obtain co-planarity with the double bond during formation of the stabilised allylic anion. The possibility of the organolithium reagent being coordinated to the carbamate group through oxygen and nitrogen and thereby being rendered unreactive, cannot be excluded.

2.4.2. Synthesis of *N*-tosyl ketene-*S*,*S*-acetal **103**.

The method used to prepare *N*-tosyl ketene-*S*,*S*-acetal **103** is outlined in figure 50. Vinyl carbamate **97** was obtained as described before (section 2.4.1.). Acidic hydrolysis liberated the secondary amine which was immediately reprotected, without purification, as the *N*-tosyl derivative **45** in 58% yield. Peterson olefination with 2-lithio-2-trimethylsilyl-1,3-dithiane was essentially complete once addition of the reagent was completed at -78°C. A 61% yield of *N*-tosyl ketene-*S*,*S*-acetal **103**

was realised after recrystallisation.

The nitrogen of sulphonamides is not as basic as the nitrogen of amines due to the strong inductive electron withdrawing nature of the sulphone group. As expected, because of these effects, sulphonamides **45** and **103** were less polar and better resolved by chromatography on silica gel than their tertiary amine analogues.

When sulphonamide **103** was treated with n-butyllithium and iodomethane using the conditions developed for alkylation of the *N*-methyl analogue (section 2.3.), a 24% yield of reclaimed starting material **103** was obtained. In addition two other very polar components were also formed. ^{1}H n.m.r. (270MHz) of these components did not indicate the presence of a tosyl group, as was also indicated by the polarity of the compounds. Attempted isolation of these components by preparative t.l.c. led to extensive decomposition and no firm identification of the two components being made.

The sulphone group of sulphonamide **103** imparts to this compound the possibility of several alternative courses of reaction with organolithium reagents other than the desired mode of reactivity. It is known that sulphones are good *ortho*-lithiation directing groups for aromatic substrates. It is also known that sulphonamides can be cleaved to sulphones and amines in the presence of electron transfer reagents or under electrolytic conditions.[97] Organolithium reagents can react via an electron transfer pathway.[98] One possibility therefore is that under the conditions of the reaction *N*-*S* bond scission is occurring rather than allylic anion formation.

As in our hands attempts to alkylate the *N*-Boc and the *N*-tosyl substituted ketene-*S*,*S*-acetals **98** and **103** had been unsuccessful work was continued with the

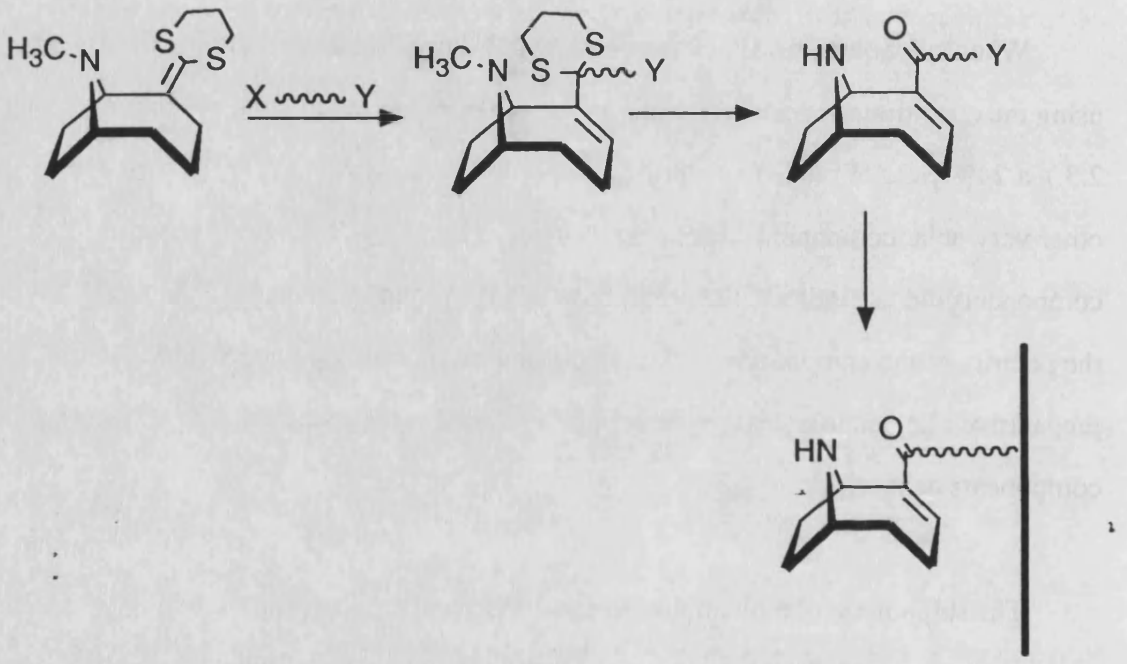


Figure 51 : C-11 functionalised derivative of anatoxin for use in affinity chromatography.

N-methyl compound **77**. This compound was the starting point for all further work described.

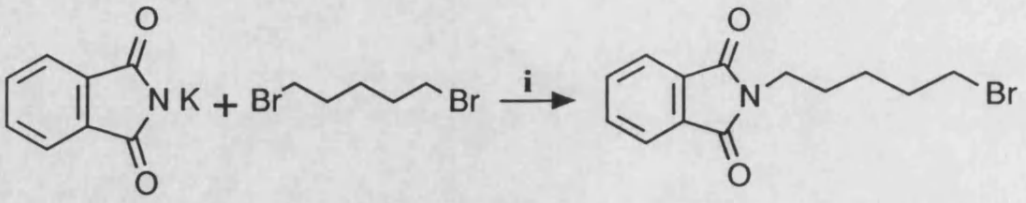
2.5. C-11 Alkyl-substituted derivatives of anatoxin.

In order to prepare derivatives of anatoxin functionalised at C-11, starting from ketene-*S*,*S*-acetal **77**, which could be immobilised by attachment to a polymeric support, we required a bifunctional reagent (figure 51). A primary alkyl halide appeared to be a suitable electrophilic partner for attachment of a spacer-arm to the allylic anion obtained from acetal **77**. Immobilisation of the alkylated material required the presence of a distal functionality on the spacer-arm which was stable to the strongly basic conditions of the alkylation and the conditions used for elaboration of the anatoxin skeleton.

Polymeric supports containing free amine and carboxyl functionalities are commercially available. Therefore, we envisaged amine or carboxylic acid groups (or their synthetic equivalents) on the spacer-arm as suitable for immobilisation of the affinity ligand by formation of an amide bond. Three different functional groups were investigated for their suitability in this respect.

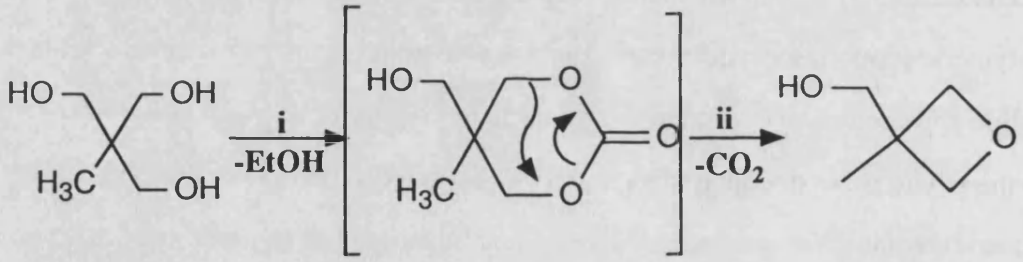
2.5.1. A functionalised phthalimide as amine precursor.

N-Alkyl phthalimides have frequently been used as protected primary amines (the Gabriel synthesis). We investigated the stability of this group to the allylic anion obtained from ketene-*S*,*S*-acetal **77**. The phthalimide used was *N*-(5-bromopentyl)-phthalimide **104**, which was made from potassium phthalimide



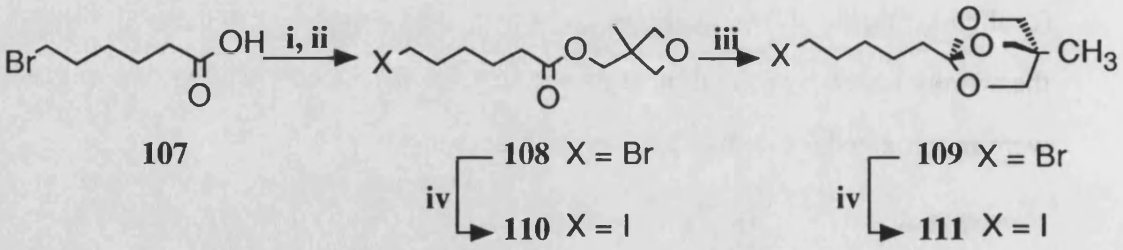
104

Figure 52 : *Reagents and conditions :* i, DMF, 150°C, 3h.



105 106

Figure 53 : *Reagents and conditions :* i, $(EtO)_2CO$, KOH, 80°C to 140°C; ii, 80°C at 40mmHg.



107 108 X = Br 109 X = Br 110 X = I 111 X = I

Figure 54 : *Reagents and conditions :* i, $SOCl_2$, reflux, 0.5h; ii, **106**, Pyr, CH_2Cl_2, 0°C, 0.75h; iii, $BF_3.OEt_2$(0.25 equiv.), CH_2Cl_2, 0°C, 2h then Et_3N; iv, NaI, $(CH_3)_2CO$, room temp., 1.5h.

and 1,5-dibromopentane by a slight modification of the procedure of Ford *et al.* (these workers reported the homologous reaction with 1,6-dibromopentane) (figure 52).[99] The anion of ketene-*S*,*S*-acetal **77** was formed and a solution of the phthalimide **104** added. During the course of the addition the reaction mixture became a very dark green/black colour. Analysis of the reaction mixture by t.l.c. indicated formation of a single new component. Attempted isolation of this component by chromatography led to extensive decomposition. However, ^{1}H n.m.r. and mass spectra of the crude product did not indicate the presence of the desired compound. This reaction was not investigated further as it was suspected that nucleophilic attack at one of the imide carbonyl groups may be competitive with displacement of bromide.

2.5.2. A functionalised ortho ester as carboxylic acid precursor.

A suitable route to a class of usable bridged ortho esters from carboxylic acids as been reported by Corey and Raju.[100] Bridged ortho esters derived from esters of 3-methyl-3-hydroxymethyloxetane **106** are especially useful because of their chromatographic stability as compared to acyclic ortho esters.
3-Methyl-3-hydroxymethyloxetane **106** was prepared from the commercially available and inexpensive triol **105** (figure 53) by a previously described method.[101] Esterification of 6-bromohexanoic acid **107** with alcohol **106** was accomplished after prior activation of the acid as the acyl chloride. The ester **108** was obtained in 86% yield (figure 54). Rearrangement of oxetane ester **108** to bridged ortho ester **109** occurred under Lewis acid catalysis with boron trifluoride etherate in 57% yield. Yields of the rearranged product were decreased if the boron trifluoride etherate was not distilled *immediately* before use.

The rearrangement presumably occurs by initial coordination of the oxetane

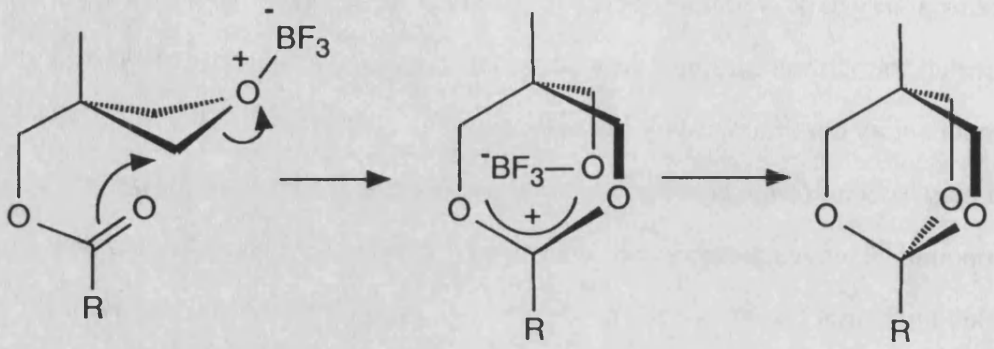
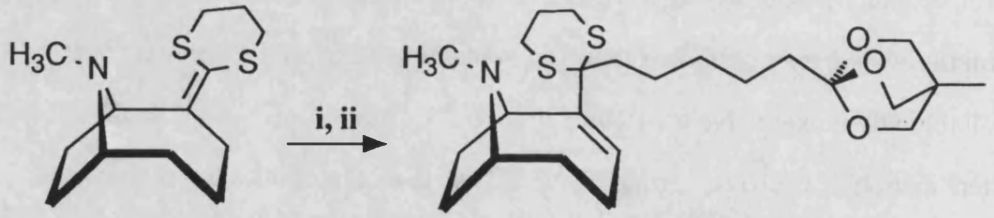


Figure 55 : Rearrangment of oxetane ester to bridged orthoester under Lewis acid catalysis.



77 **112**

Figure 56 : *Reagents and conditions :* i, Bu^{n}Li, THF, -78°C then room temp., 3h; ii, **111**, -78°C, 0.5h.

oxygen to the Lewis acid (figure 55). This induces heterolysis of the oxetane C-O bond with ester carbonyl participation. The intermediate zwitterion collapses to give the desired ortho ester. The thermodynamic driving force for rearrangement is clearly the release in ring strain on fragmenting the oxetane ring.

The electrophilicity of the alkyl halide **109** was increased by conversion to the alkyl iodide **111**, under Finkelstein conditions, in 76% yield (figure 54). Iodo ortho ester **111** could also be obtained by conversion of the bromo ester **108** to the iodo ester **110** under Finkelstein conditions in 78% yield. Oxetane ester **110** also rearranged cleanly under Lewis acid catalysis to the iodo ortho ester **111** in 74% yield. This second route avoided unnecessary handling of the acid labile ortho ester group.

The allylic anion of ketene-*S*,*S*-acetal **77** was formed under standard conditions (section 2.3.). Alkylation of the resulting allylic anion with iodo ortho ester **111** was rapid at -78°C, the reaction being complete within 15 min. A 71% yield of substituted 1,3-dithiane **112**, the product arising from alkylation α to the 1,3-dithiane ring was obtained (figure 56). There was no evidence for any reaction having occurred at the γ position of the allylic anion.

Preparation of a derivative of *N*-methyl anatoxin substituted at C-11, the methyl ketone position, was attempted. Simultaneous acid-mediated hydrolysis of the 1,3-dithiane and ortho ester groups was investigated with aqueous hydrochloric acid in *p*-dioxane both with and without DMSO. In both cases a very polar component was formed which was initially thought to be the zwitterionic form of the amino acid. However, attempted isolation of this component by HPLC led to extensive decomposition.

N-Demethylation of the alkylated 1,3-dithiane **112** was attempted, as a potential route to a derivative of anatoxin substituted at C-11. Attempts to form the vinyl carbamate derivative with vinyl chloroformate failed. Initially this was ascribed to hydrolysis of the chloroformate, by trace amounts of moisture,the hydrogen chloride released forming the hydrochloride salt of the tertiary amine and rendering the amine nitrogen non-nucleophilic. However, repeating the reaction in the presence of molecular sieves (to remove water), anhydrous potassium carbonate (to neutralise any hydrogen chloride formed) and under an atmosphere of dry nitrogen failed to promote the desired reaction. Attempted acceleration of the reaction by using refluxing 1,2-dichloroethane (b.p. 83°C) in place of methylene chloride (b.p. 40°C) was also unsuccessful. Our lack of success with this reaction may have been due to the competing reaction pathway (path b in figure 47) now being the preferred mode of reaction.

Due to our inability to elaborate the alkylated 1,3-dithiane **112** by *N*-demethylation or by hydrolysis without causing decomposition our studies of **112** as a potential precursor for C-11 substituted derivatives of anatoxin and *N*-methyl anatoxin were abandoned.

2.5.3. An alkene as a carboxylic acid precursor.

It was envisaged that an alkyl halide with a terminal alkene substituent would be a suitable electrophile for the alkylation of the allylic anion obtained from ketene-*S*,*S*-acetal **77**. It was anticipated that the alkenyl group in the spacer-arm would be stable to the basic conditions of the alkylation, the acidic conditions of 1,3-dithiane hydrolysis and the conditions used for *N*-demethylation.

The allylic anion of ketene-*S*,*S*-acetal **77** was formed using the conditions we

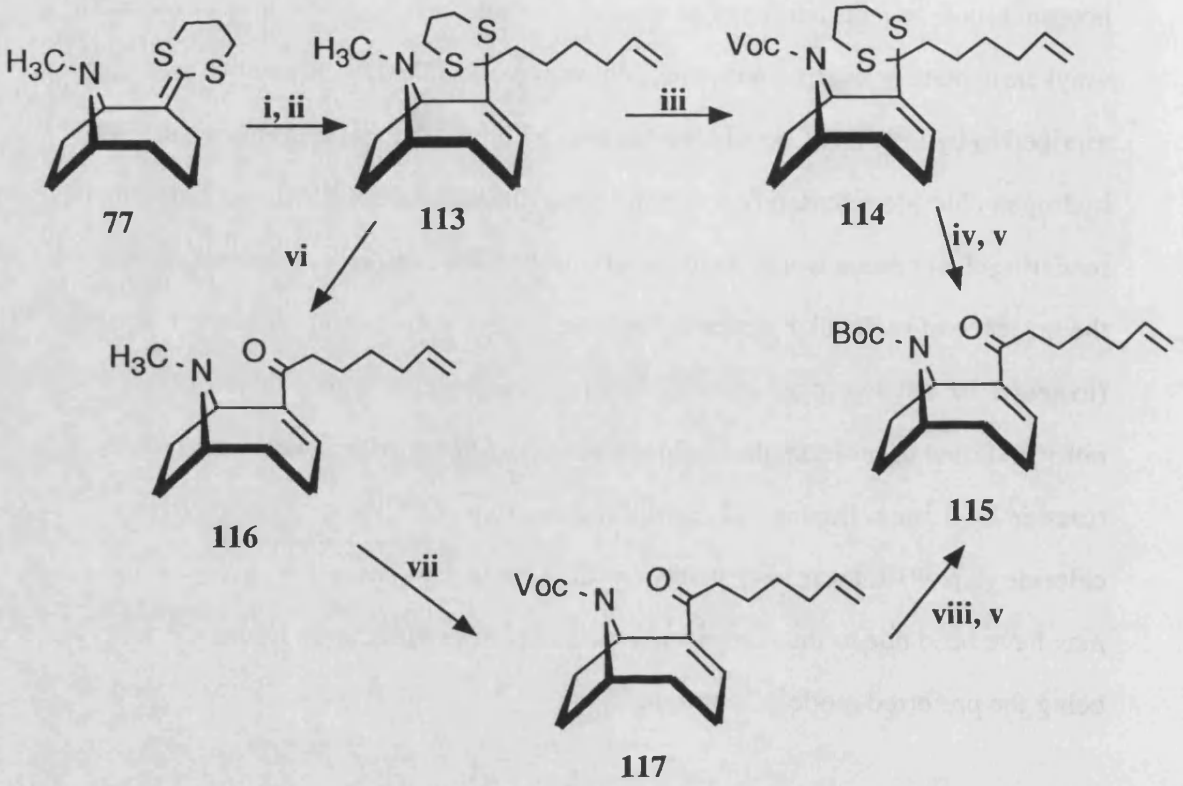
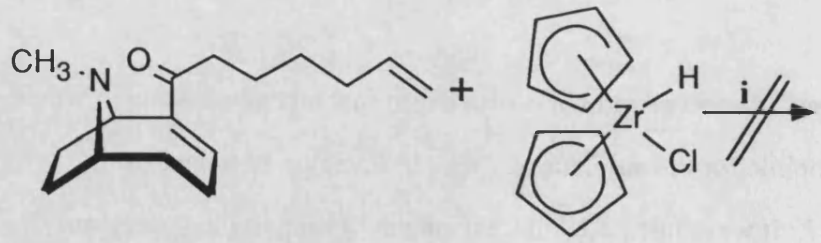


Figure 57 : *Reagents and conditions :* i, Bu^nLi, THF, -78°C then room temp., 3h; ii, 6-bromohexene, THF, -78°C, 30min; iii, vinyl chloroformate, CH_2Cl_2, reflux, 20h; iv, 3.6M $HCl_{(aq)}$, *p*-dioxane, reflux, 8h; v, $(Boc)_2O$, Et_3N, THF/H_2O 2:1, room temp., 16h; vi, 3.6M $HCl_{(aq)}$, *p*-dioxane, reflux 20h; vii, vinyl chloroformate, CH_2Cl_2, reflux, 16h; viii, 3.6M $HCl_{(aq)}$, *p*-dioxane, reflux, 2h.



116 118

Figure 58 : *Reagents and conditions :* i, CO (1 atm.), C_6H_6, room temp., 24h then Br_2/MeOH.

had developed previously (section 2.3.) and was trapped with 6-bromo-1-hexene to give the substituted 1,3-dithiane **113** in 92% yield (figure 57). No product arising from alkylation γ to the dithiane ring was observed. α-Alkylation was indicated by a ^{1}H n.m.r. resonance at δ_H 6.20 due to the alkenyl proton attached to the bicyclic skeleton. The protons of the terminal alkenyl group appeared as an AB_2 multiplet in the ^{1}H n.m.r spectrum. The terminal protons were a two proton multiplet at δ_H 4.88-5.06 and the internal proton as a one proton multiplet at δ_H 5.70-5.89. ^{13}C n.m.r. indicated the presence of four alkenyl carbon nuclei, which 95 and 135 DEPT experiments showed were a terminal alkenyl carbon, two mono-substituted alkenyl carbons and one disubstituted carbon nuclei in accord with the proposed structure, **113**.

It was initially envisaged that the terminal alkene group of **113** could be converted to a methyl ester using Schwartz's hydrozirconation procedure followed by oxidation with bromine in methanol.[102] Alkylated 1,3-dithiane **113** was hydrolysed with aqueous acid to the corresponding α,β-unsaturated ketone **116** (figure 57). Addition of zirconocene hydrochloride **118** to primary alkene **116** followed by stirring under carbon monoxide at atmospheric pressure before adding bromine in methanol failed to produce any methyl ester-containing products (figure 58). Other successful reports of this type of reaction have involved the use of carbon monoxide pressures up to 20 atmospheres. Reaction under these conditions was not investigated. It was also possible that coordination of zirconium by the tertiary methylamine was taking place thereby preventing reaction with the alkene.

Alternatively, it was anticipated that the terminal alkene group could be converted into a carboxylic acid derivative by ozonolysis. This required the selective ozonolysis of the acyclic alkene in the presence of the cyclic alkene. As it was intended to use ozone to functionalise the terminal alkene it was necessary to

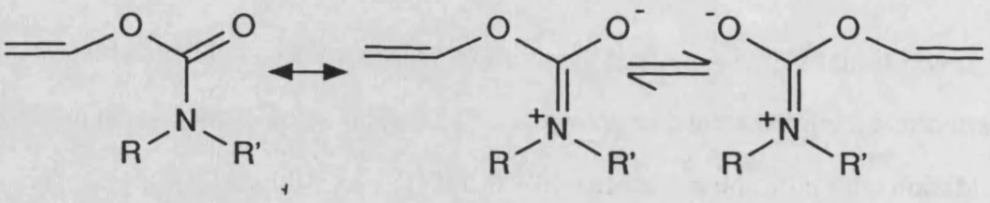


Figure 59 : *Cis -trans* double bond equilibria for vinyl carbamate.

protect the nitrogen against electrophilic attack from ozone, resulting in *N*-oxide formation. It has been reported that alkenes containing amines can be ozonolysed without competing *N*-oxide formation if the nitrogen is protected as an amide.[103] We opted to use a carbamate protecting group as this should be more readily removable at the end of the synthesis than the corresponding amide.

N-Demethylation of alkylated dithiane **113** was achieved with vinyl chloroformate to give vinyl carbamate **114** in 59% yield (figure 57). The ^{1}H n.m.r. of carbamate **114** was complicated due to the superimposition of corresponding signals from the *cis* and *trans* forms of the carbamate (figure 59). This indicated that significant delocalisation of the nitrogen lone pair was occurring into the carbonyl group and the nitrogen should therefore be relatively inert to electrophilic reagents such as ozone. However, carbamate **114** was not considered a suitable substrate for ozonolysis as the alkenyl group of the carbamate should be relatively electron rich because of electron donation from the ether oxygen. Therefore, the vinyl carbamate alkene group would be reactive towards ozone, possibly giving rise to unwanted products. The carbamate **114** also contained two nucleophilic sulphur atoms which could react with ozone. The cyclic alkene was only differentiated from the acyclic alkene by its different substitution pattern, degree of steric hindrance and the degree of bond angle deformation associated with the cyclic alkene.

Hydrolysis of carbamate **114** was performed with aqueous acid to liberate the secondary amine and form the α,β-unsaturated ketone simultaneously. The secondary amine was reprotected as the Boc carbamate **115** before isolation in 42% yield (25% from tertiary amine **113**). As with vinyl carbamate **114** the ^{1}H n.m.r. spectrum of Boc carbamate **115** was complicated by the presence of *cis* and *trans* forms of the carbamate. However, where signals were well resolved an intergral ratio of approximately 2:1 was measured, an indication of the relative populations of

the two carbamate configurations. The nitrogen lone pair was therefore significantly delocalised into the carbonyl group and rendered non-nucleophilic.

The cyclic alkene was in conjugation with the ketone and would therefore be stabilised with respect to the primary alkene. The sterically demanding Boc carbamate may also hinder the approach of ozone to the cyclic alkene, making reaction of the primary alkene more selective. It has also been reported that the selectivity ozone demonstrates for isolated carbon-carbon double bonds in the presence of conjugated ketones can be increased in the presence of pyridine.[104]

An alternative and higher yielding route to Boc carbamate **115** is also shown in figure 57. Hydrolysis of dithiane **113** with aqueous acid gave α,β-unsaturated ketone **116** in 76% yield. *N*-Demethylation of tertiary amine **116** was accomplished with vinyl chloroformate to give vinyl carbamate **117** in 79% yield. ^{1}H n.m.r. spectroscopy with this compound indicated an approximately 5:3 ratio of carbamate conformational isomers. Acid hydrolysis released the secondary amine which was protected as the Boc carbamate **115** prior to isolation in 76% yield (46% from dithiane **113**).

Ozonolysis of primary alkene **115** was performed in glacial acetic acid. Removal of the Boc group was not observed as acetic acid is not a strong enough acid to protonate the carbamate carbonyl group, leading to alkyl-oxy fission and liberation of the secondary amine. Ozonolysis was performed by addition of aliquots of ozone in a stream of oxygen to a solution of primary alkene **115** and the reaction monitored by t.l.c. for the amount of alkene **115** remaining in solution.

Since this reaction was performed it has been suggested that the controlled addition of one equivalent of ozone may be possible in the presence of an alkene-

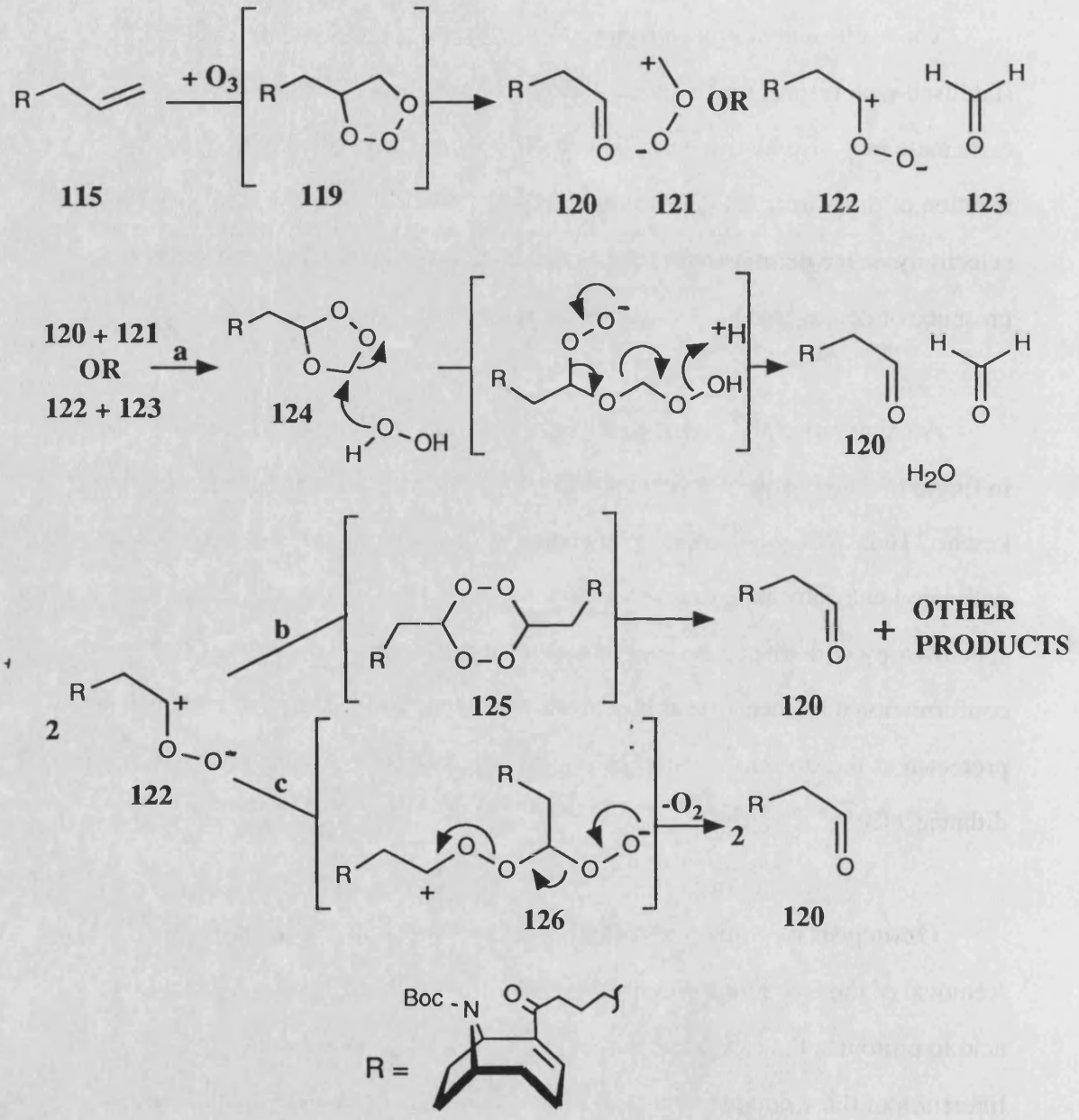


Figure 60 : Possible mechanisms for formation of aldehyde **120** by ozonolysis under non-oxidising conditions.

containing dye.[105] This procedure relies on the selection of a dye containing alkene groups with reactivity intermediate between that of the substrate alkene group whose selective ozonolysis is desired and the substrate alkene group whose ozonolysis is to be avoided. Decolourisation of the dye indicates that ozonolysis of the most reactive alkene group is complete.

Oxidation of the ozonide directly to the carboxylic acid was attempted with hydrogen peroxide followed by treatment with diazomethane to convert any carboxylic acid formed to the corresponding methyl ester. A 37% yield of the aldehyde **120**, arising from fragmentation of the ozonide **119** without oxidation, was obtained.

Analysis of the Criegee intermediates formed on decomposition of the primary ozonide **119** show how the aldehyde **120** may have arisen (figure 60). Primary ozonide **119** is unstable and will fragment to one or other of the two sets of products **120** and **121** or **122** and **123**. Although **121** and **122** are shown as zwitterions they may also be biradicals. If either of these pairs recombine (path a) the ozonide **124** will be formed which upon hydrolysis without oxidation can give aldehyde **120**, formaldehyde, and water. The aldehyde **120** may also arise more directly if the hydroperoxide **121** couples with itself.

From the alternative pair of primary ozonide products the alkyl hydroperoxide **122** may dimerise in one of two ways. If diperoxide **125** is formed (path b) hydrolysis can give aldehyde **120** along with other products. The alternative mode of dimerisation (path c), to give the zwitterionic diperoxide **126**, will result in formation of two moles of aldehyde **120** on loss of oxygen.

In our hands oxidation of the ozonolysis product with periodic acid in

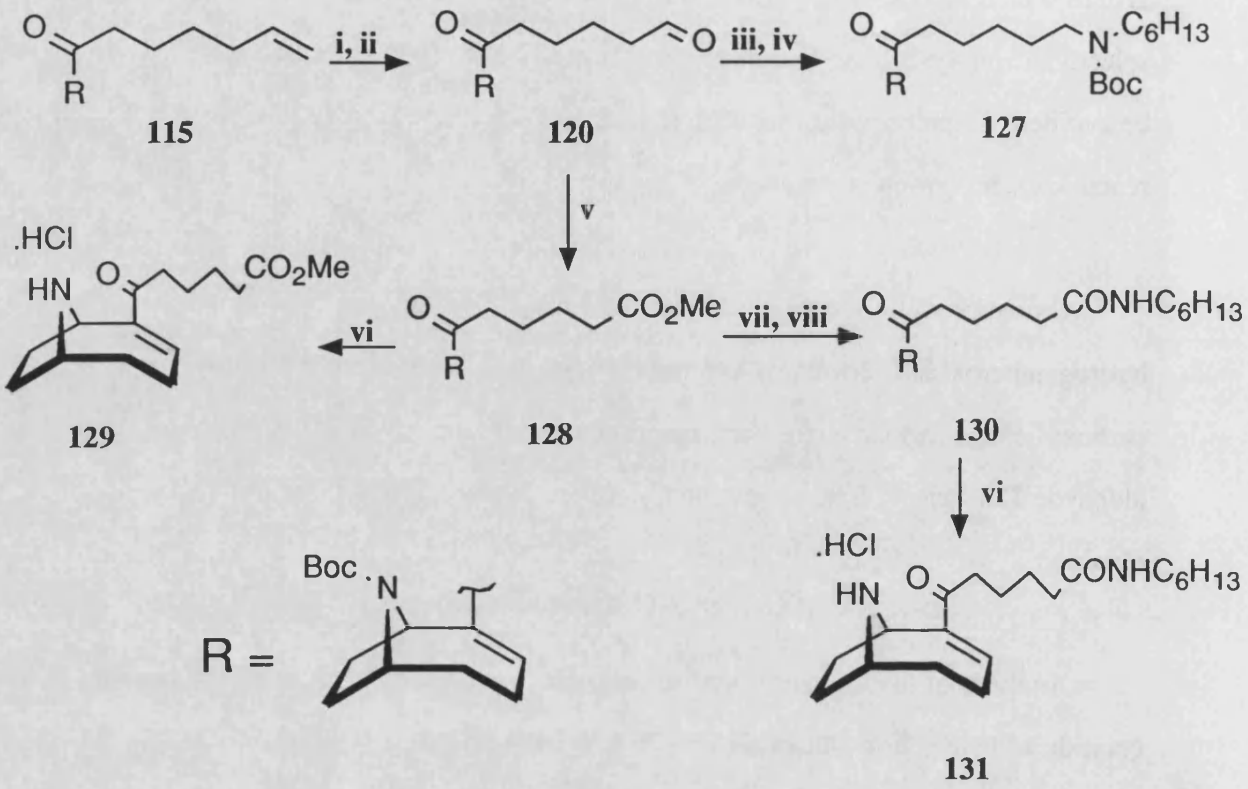


Figure 61 : *Reagents and conditions :* i, O_3, CH_2Cl_2, -78°C; ii, PPh_3, -78°C to room temp.; iii, $^nC_6H_{13}NH_2$, $NaCNBH_3$, HCl, MeOH, room temp., 1h; iv, $(Boc)_2O$, Et_3N, THF/H_2O 2:1; v, Br_2, MeOH, $NaHCO_3$; vi, CF_3CO_2H, 0°C, 10min; vii, LiOH (2 equiv.), THF/H_2O, room temp., 6h then H^+; viii, $^nC_6H_{13}NH_2$, DCC, CH_2Cl_2, room temp..

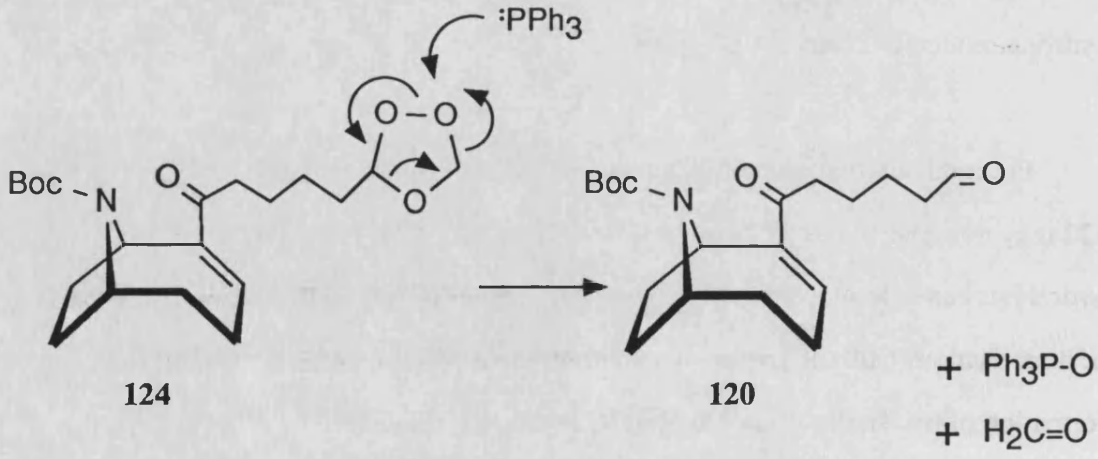


Figure 62 : Reductive fragmentation of ozonide **124** with triphenylphosphine to give aldehyde **120**.

methylene chloride or glacial acetic acid was unsuccessful.[106] A plethora of components was formed in both cases. Isolation and identification of these products was not attempted.

Reductive fragmentation of ozonide **124** to give aldehyde **120** was achieved in 51% yield on treatment with triphenylphosphine (figure 61). Phosphine reductions of ozonides have been shown to occur, using ^{18}O labelling experiments, via attack of phosphorus on one of the peroxidic oxygens, probably the external oxygen on steric grounds (figure 62).[107] The driving force for the reaction is formation of the strong phosphorus-oxygen bond (500-630 $KJmol^{-1}$). When this fragmentation was attempted using dimethylsulphide in methanol only a 26% yield of aldehyde **120** was obtained. This may be a reflection of a different reaction pathway being involved under these conditions, an α-methoxyalkyl hydroperoxide being formed by attack of methanol on ozonide **124**.

We wished to show that aldehyde **120**, and more generally that an alkene substituted spacer-arm, could be used for the preparation of affinity ligands based on anatoxin. In the real affinity ligand this would require the attachment to a polymeric support containing free amine groups. Rather than attachment to a polymeric support we used a simple amine (n-hexylamine) as a model for the polymeric support.

Aldehyde **120** underwent reductive amination with n-hexylamine in the presence of methanolic hydrogen chloride and sodium cyanoborohydride (figure 61). Sodium cyanoborohydride is stable in acid media down to pH 3 and can be used to selectively reduce imines selectively in the presence of ketones.[108] The secondary amine formed was converted to the corresponding Boc carbamate **127** before isolation in 13% yield. Removal of the Boc group was attempted with

trifluoroacetic acid. All of the bis-Boc carbamate **127** was consumed within 5min but none of the expected diamine could be isolated. While the low yield obtained from the reductive amination may be attributed to competing intramolecular reaction between the spacer-arm amine and the α,β-unsaturated ketone our failure to isolate the diamine was a surprise. It would be expected that in the presence of trifluoroacetic acid the amine groups, once liberated from the Boc carbamates, would be completely converted to the protonated form and rendered non-nucleophilic.

Aldehyde **120** was oxidised with bromine in methanol according to the procedure of Williams *et al.* to give methyl ester **128** in 24% yield.[109] The Boc group was removed using trifluoroacetic acid and the resulting secondary amine converted to the hydrochloride salt **129** in near quantitative yield. The methyl ester group of Boc carbamate **128** was hydrolysed with lithium hydroxide to give the free acid after acidification. Amide formation with n-hexylamine was accomplished with DCC under standard coupling conditions to give amide **130** in 64% yield. Boc carbamate **130** was deprotected to liberate the secondary amine upon treatment with trifluoroacetic acid. The secondary amine was isolated as the corresponding hydrochloride salt **131** in 26% yield. Conversion of aldehyde **120** through to hydrochloride salt **131** has as yet only been performed on a small scale and yields are unoptimised. It is hoped that further investigation the reactions involved will lead to a significant improvement in overall yields.

This constitutes the synthesis of a suitable model of a C-11 functionalised derivative of anatoxin for affinity chromatography. We have shown that an alkene bearing spacer-arm can be introduced at C-11 and that the alkene group is stable to the conditions used to elaborate the anatoxin skeleton. When protected as the Boc carbamate the bicyclic ligand is stable to the conditions used to elaborate the alkene

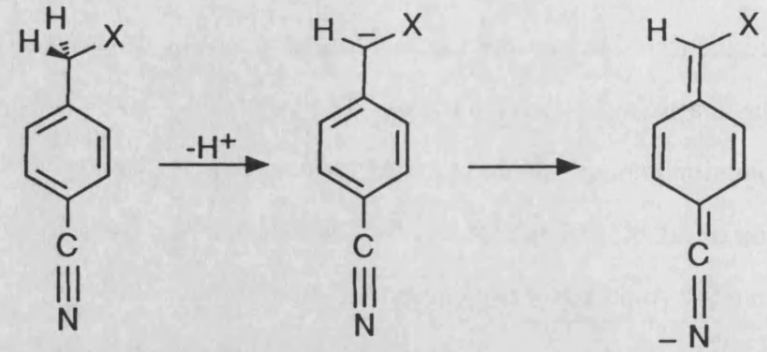
into a functional group suitable for coupling to a polymeric support.

The introduction of spacer-arms containing alkene substituents at different positions of the anatoxin skeleton should also be elaborable in a similar manner. Reductive amination is not a suitable method for coupling as decomposition occurs on deprotection of the bicyclic amine and the secondary amine in the spacer-arm. It may be possible to avoid this by use of a protecting group for the spacer-arm amine which is not removed under the conditions used to liberate the bicyclic amine from the Boc carbamate.

2.6. C-11 Phenyl-substituted derivatives of *N*-methyl anatoxin.

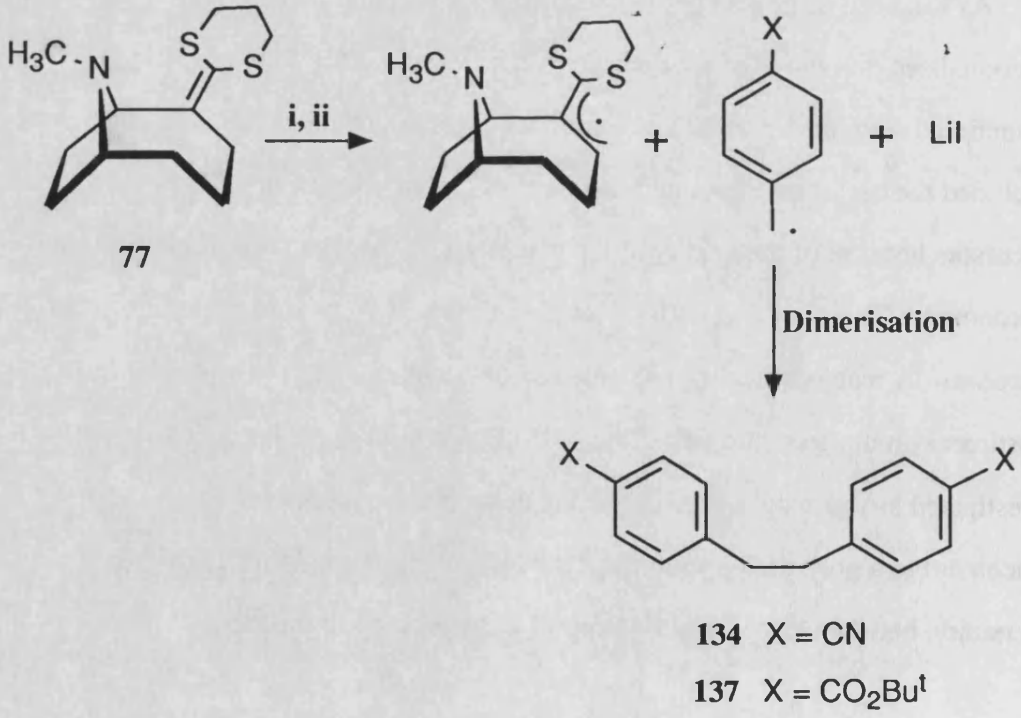
As has been discussed before (section 2.5.), preparation of a C-11 functionalised derivative of anatoxin from ketene-*S*,*S*-acetal **77** required the use of a bifunctional reagent (figure 51). Use of a simple alkyl chain as a spacer arm precluded the use of ester and nitrile groups as carboxylic acid and/or amine precursors because of the acidity of the α-protons. However, this problem can be overcome by the use of aryl carboxylates or nitriles as these substrates contain no α-protons. A reactive electrophilic site can be accommodated as a halomethyl substituent on the aromatic ring. The *p*-disubstituted benzene compounds were investigated as this would provide the greatest separation between the point of attachment to a polymeric support and the ligand, thereby minimising steric interaction between the polymeric support and the receptor protein.

The allylic anion of ketene-*S*,*S*-acetal **77** was formed using the conditions we had developed (section 2.3.) and trapping was attempted by the addition of



132 X = Br
133 X = I

Figure 63 : Mesomeric and inductive stabilisation of benzylic carbanion by bromine and nitrile groups.



77

134 X = CN
137 X = CO_2Bu^t

Figure 64 : i, Bu^nLi, THF, -78°C then room temp., 3h; ii, **133** or **136**, -78°C.

4-cyanobenzyl bromide **132**. The ketene-*S*,*S*-acetal **77** was the only isolable product and was obtained in 52% yield. Although the double bond in the isolated product had not moved into the ring, as in the cyclic alkene **96** obtained by trapping the anion with saturated aqueous ammonium chloride (figure 44), it was still presumed that anion formation had occurred. The allylic anion may have abstracted a proton from the benzylic halide, the resulting benzylic anion being stabilised by the mesomeric and inductive effects of the *p*-nitrile group and the inductive effect of the adjacent bromine (figure 63). The steric interaction between the allylic anion and the benzylic substrate will favour proton abstraction at the less hindered γ-position.

The reaction was repeated using 4-cyanobenzyl iodide **133**, obtained from benzyl bromide **132** by Finkelstein reaction. When benzylic iodide **133** was added to the allylic anion all of the benzylic substrate was consumed within 30 min, as judged by t.l.c.. Along with several polar components, which could not be isolated pure, a new non-polar and fluorescent compound was formed in 23% yield. This was identified as 4,4′-dicyanobibenzyl **134** by ^{1}H n.m.r. and high resolution accurate mass determination (figure 64).[109a]

It may be that bibenzyl **134** is formed by reaction of benzyl iodide **133** with the slight excess of n-butyllithium (1.2 equivalents used) still present in the reaction mixture. It is known that bibenzyl can be formed in high yield by the reaction of n-butyllithium on benzyl bromide.[110] Alternatively reaction may occur with the allylic anion. In either case such reactions are known to proceed via electron transfer processes involving the formation of relatively stable benzyl radicals (figure 63).[111] The allylic radicals invoked by this rationale could undergo a number of reactions including combination with other radical species, homolytic reaction with non-radical species and possibly rearrangement. This alternative homolytic reaction pathway is implicated by the isolation of bibenzyl **134**.

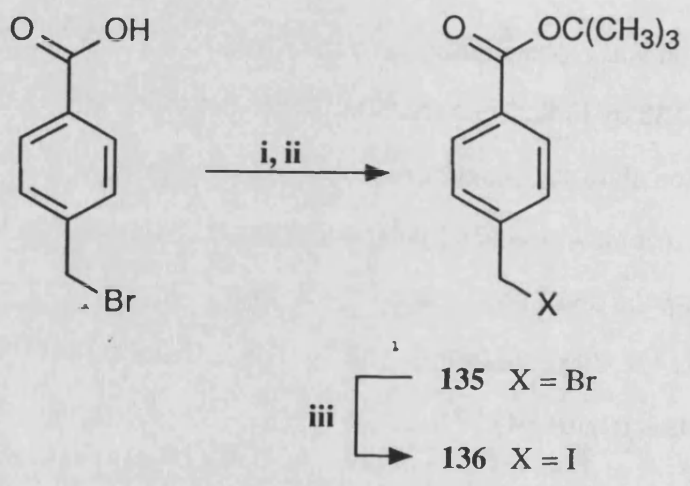
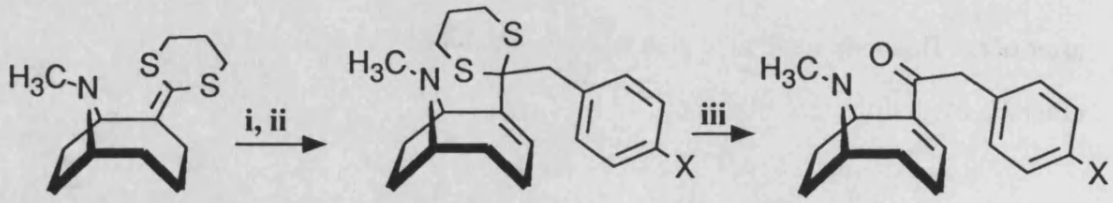


Figure 65 : *Reagents and conditions :* i, $SOCl_2$, reflux, 1h; ii,$HOC(CH_3)_3$, Bu^nLi (1 equiv.), Et_2O, room temp., 16h; iii, NaI (5 equiv.), $CO(CH_3)_2$, room temp., 1h.

The alkylation reaction was also investigated with the 4-*t*-butyl ester substituted benzyl bromide **135** and iodide **136**. These esters were prepared by the reaction of lithium *t*-butoxide with 4-bromomethylbenzoyl chloride (figure 65).[112] Substituted benzyl bromide **135** was obtained in 74% yield by this method and was converted to the benzyl iodide **136** by Finkelstein reaction in 90% yield. Formation of the *t*-butyl benzoate by sulphuric acid catalysed reaction of an ethereal suspension of the acid with 2-methylpropene in a sealed tube gave only a 17% yield of the ester after 66h. This may have been due to the low solubility of the acid in the diethyl ether / 2-methylpropene mixture.

Addition of substituted benzyl bromide **135** to a solution of the anion of ketene-*S*,*S*-acetal **77** resulted in formation of several minor components which could not be isolated pure and a major component which was co-running by t.l.c. with the original acetal **77**. The ^{1}H n.m.r. spectrum of this compound indicated that it was mainly impure acetal **77**. With the more electrophilic benzyl iodide **136** a small amount of the 4,4′-bis(*t*-butyl ester) substituted bibenzyl **137** was isolated. This presumably arose in a manner similar to that for the formation of bibenzyl **134**.

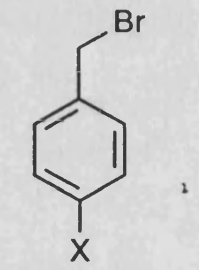
The above results showed that the benzyl halides **132, 133, 135**,and **136** had an alternative mode of reactivity to that desired. Benzylic radical and/or anion formation was occurring in preference to nucleophilic displacement of halide, probably due to mesomeric stabilisation by the *p*-nitrile and ester groups. We envisioned that use of cerium(III) in place of lithium(I) as the counter-ion of the allylic anion may lead to the desired mode of reactivity. Cerium(III) reagents are considered to be less basic and more nucleophilic than their corresponding lithium and Grignard reagents.[113] This effect with carbonyl compounds has been ascribed to the greater oxaphilicity of cerium(III) compared with lithium(I) and



77

139 X = Br
142 X = I

140 X = Br
143 X = I



138 X = Br
141 X = I

Figure 66 : *Reagents and conditions :* i, Bu^nLi, THF, -78°C then room temp., 3h; ii, **138** or **141**, THF, -78°C, 0.5h; iii, 3.6M $HCl_{(aq)}$, *p*-dioxane, reflux, 16h.

magnesium(II). Trans-metallation was performed as described by Imamoto, by the addition of the lithium-allylic anion to a suspension of anhydrous cerium(III) chloride in THF.[114] Addition of benzyl iodide **136** to this mixture yielded 39% of the acetal **77** as the only isolable material by chromatography.

To avoid the unwanted reactions encountered with benzyl halides substituted with mesomerically stabilising groups the reactions of 4-halogeno substituted benzyl halides was investigated. We envisaged that the aryl halide could later be converted to an aryl ester by transition metal-mediated carbonylation.

Addition of 4-bromobenzyl bromide **138** to the anion of ketene-*S*,*S*-acetal **77** resulted in essentially instantaneous reaction at -78°C, the substituted 2-benzyl-1,3-dithiane **139** being formed in 78% yield (72% conversion) (figure 66).

Before attempting palladium(0) catalysed aryl halide carbonylation, hydrolysis of the 1,3-dithiane was performed. This was carried out prior to attempting the carbonylation as it was presumed that the sulphur atoms of the 1,3-dithiane ring would chelate the transition metal and render the catalyst inactive. Many methods have been published for the release of carbonyl functionality from 1,3-dithianes. Some of these methods were evaluated for conversion of 1,3-dithiane **139** to α,β-unsaturated ketone **140**.

Recently Stork *et al.* have reported the use of bis(trifluoroacetoxy)-iodobenzene for the liberation of ketones from 1,3-dithianes.[115] This procedure is reported to be well suited for use in the presence of amines as the nitrogen is protonated under the conditions of the reaction (by the release of trifluoroacetic acid) and is therefore rendered inert to condensation with the ketone generated. When substituted 1,3-dithiane **139** was treated with bis(trifluoroacetoxy)-

iodobenzene low yields of impure α,β-unsaturated ketone **140** were obtained. Further attempts to purify this ketone were unsuccessful in our hands, the main component undergoing extensive decomposition.

A well established method of 1,3-dithiane hydrolysis which was investigated involved hydrolysis with mercury(II) oxide and tetrafluoroboric acid.[116] Under these conditions all of substituted 1,3-dithiane **139** was consumed within 2h at room temperature and a single new component formed, as judged by t.l.c.. However, the infrared spectrum of this compound did not exhibit an α,β-unsaturated ketone stretching band. No further analysis of this compound was carried out as it could not have been the desired compound.

Thallium(III) nitrate is another heavy metal thiophile which has been used for dethioketalisation.[117] In particular this reagent appeared promising because it had been successfully used for the hydrolysis of 2-vinyl-1,3-dithianes to α,β-unsaturated ketones and has been used in systems containing unprotected amines. With thallium(III) nitrate substituted 1,3-dithiane **139** yielded two new compounds. One component showed no evidence for the presence of a carbonyl group from its infrared spectrum. The second compound did exhibit a carbonyl stretching frequency but no alkene proton resonance was observed by ^{1}H n.m.r.. Neither compound could therefore be the desired α,β-unsaturated ketone **140** and so no further work was undertaken with these compounds.

Finally we investigated the hydrolysis of substituted 1,3-dithiane **139** under acidic conditions. When 1,3-dithiane **139** was treated with aqueous hydrochloric acid in *p*-dioxane at reflux a 90% yield of α,β-unsaturated ketone **140** was obtained after 66% hydrolysis of 1,3-dithiane **139**. The α,β-unsaturated ketone exhibited a strong carbonyl stretching frequency at 1665cm^{-1} whilst ^{1}H n.m.r. and mass spectra

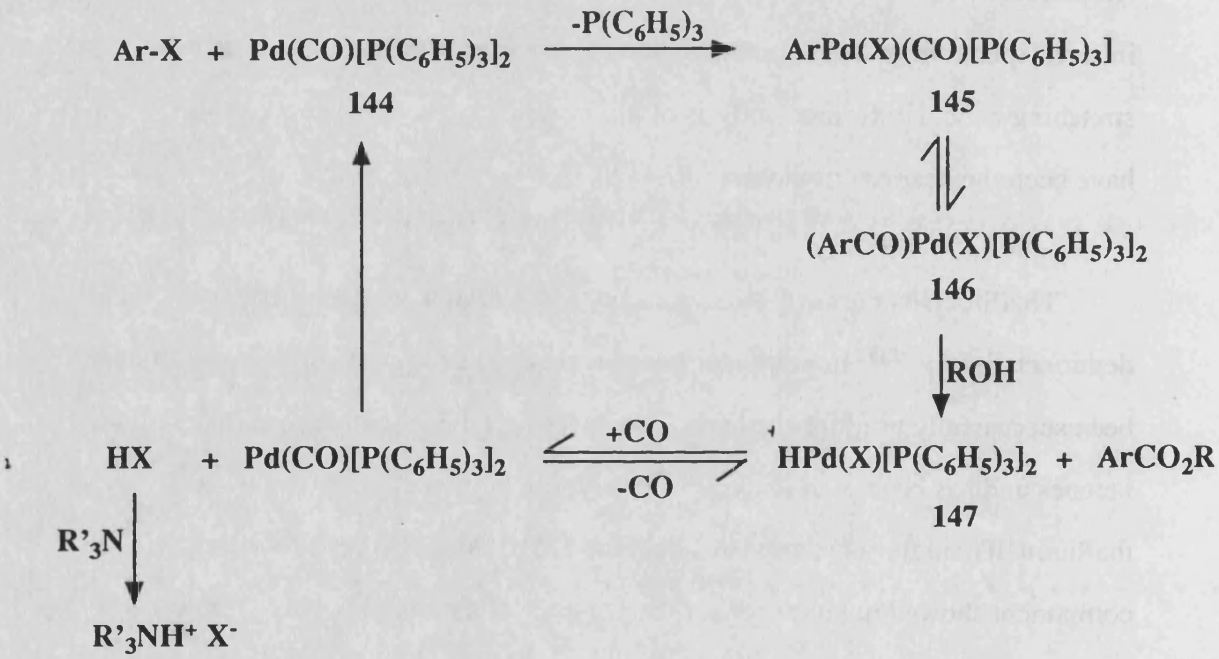


Figure 67 : Proposed catalytic cycle for palladium catalysed alkoxycarbonylation of aryl halides.

attested to the loss of the dithiane ring and the presence of one alkene proton.

With a successful dethioketalisation procedure available the remaining synthetic challenge was conversion of aryl bromide **140** to a carboxylic acid derivative. The palladium catalysed carboalkoxylation of aryl halides is a reaction which has been extensively studied by Heck and co-workers.[118] They have shown that aryl halides can react in the presence of carbon monoxide, an alcohol, a tertiary amine and a palladium catalyst to form aryl esters in good yield. The catalytic cycle has been suggested to be as shown in figure 67.

The catalytically active palladium(0) species **144** is coordinatively unsaturated and can undergo oxidative addition to the aryl-halogen bond with loss of a phosphine ligand to form the palladium(II) species **145**. Organopalladium compounds are known to undergo carbon monoxide insertion reactions very rapidly, in this case the acyl-palladium species **146** being formed. Attack of alcohol on the acyl group liberates the aryl ester and forms a hydrido-palladium(II) species **147**. Reductive elimination of HX (which is removed from the equilibrium by reaction with the tertiary amine present) and coordination to carbon monoxide reforms the catalytic palladium(0) species **144**.

The catalyst used was dibromo-bis(triphenylphosphine) palladium(II) which was generated *in situ* from an excess of potassium bromide and dichloro-bis(triphenylphosphine) palladium(II). When aryl bromide **140** was heated to reflux in methanol and triethylamine under carbon monoxide at 1 atmosphere and in the presence of this catalyst no ester formation was observed. All that could be observed by t.l.c. was unreacted aryl bromide **140** and some very polar baseline material which may have been due to the catalyst or decomposition of the aryl bromide. ^{1}H n.m.r. of the crude reaction mixture showed no evidence for methyl

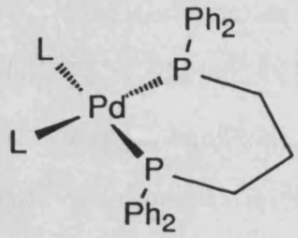


Figure 68 : Enforced *cis*-arrangment of ligands **L** in the presence of a chelating phosphine ligand.

ester formation.

Problems encountered in the palladium catalysed carboalkoxylation of aryl halides which have been reported include moderate yields, lengthy reaction times and poor catalyst turnover, even with high carbon monoxide pressures. However, the use of 1,3-bis(diphenylphosphino)propane as a chelating ligand has been reported to increase the rate of carbonylation 500-fold relative to triphenylphosphine.[119] The rate increase observed with this bidentate ligand has been ascribed to the obligatory *cis*-arrangement of the two donor atoms (figure 68). The more stable *trans*-isomer (because of relaxation of steric compression between the two diphenylphosphino groups) is not accessible because of the constraining propyl chain. However, the *trans*-isomer is unreactive as carbon monoxide insertion into the palladium-aryl bond can only occur when the two phosphine groups are *cis*, and the carbon monoxide and aryl groups also *cis*. The use of polar solvents *e.g.* DMSO or DMF, is also reported to cause a rate enhancement by increasing the degree of catalyst turnover.

Carbomethoxylation of aryl bromide **140** was attempted at 80°C in DMSO with methanol, triethylamine, palladium(II) acetate and 1,3-bis(diphenylphosphino)-propane under carbon monoxide at atmospheric pressure for 14h. Several new components were observed by t.l.c. but the crude product showed no evidence for methyl ester formation by infrared spectroscopy. In our hands carbomethoxylation of aryl bromide **140** was also unsuccessful when carried out under 100 p.s.i. pressure of carbon methoxide at 70°C for 22h.

An alternative method for obtaining aryl carboxylic acid derivatives from aryl halides requires the use of tetracarbonylnickel(0) (**Warning : *carcinogenic! highly toxic!***).[120] Under phase transfer catalysis in the presence of tetracarbonylnickel(0),

carbon monoxide and calcium hydroxide, aryl bromides can be converted to the calcium salt of the corresponding carboxylic acid. In our hands when aryl bromide **140** was exposed to these conditions at 110°C for 20h a multicomponent mixture was formed which showed no sign of the presence of carboxylate.

Our inability to convert aryl bromide **140** to a methyl ester under catalytic carbomethoxylation conditions may have been a reflection of the inherent relatively low reactivity of aryl bromides under these conditions. However, aryl iodides are more labile towards carboalkoxylation, generally being up to two orders of magnitude more reactive. The corresponding aryl iodide **143** was obtained from 4-iodobenzyl bromide **141** and ketene-*S*,*S*-acetal **77** in 81% overall yield in a manner similar to that used for the preparation of aryl bromide **140** (figure 66).

Carboalkoxylation of aryl iodides can be catalysed by iodophenyl-bis(triphenylphosphine) palladium(II). This catalyst is available from tetrakis(triphenylphosphine) palladium(0) by the oxidative addition of iodobenzene.[121] Carbomethoxylation of aryl iodide **143** was investigated with this catalyst using methanol containing triethylamine under carbon monoxide at atmospheric pressure. No evidence for methyl ester formation was found after heating to reflux for 72h. No reaction was observed with palladium(II) acetate / 1,3-bis(diphenylphosphino)propane as catalyst in DMSO after 16h at 100°C.

In all of the cases studied it was considered that carbonylation may not have been occurring because of poisoning of the palladium catalyst by sulphur containing compounds carried through from the hydrolysis of dithianes **139** and **142**. However, this was unlikely as both aryl halides gave correct elemental microanalyses and the catalyst was typically used in a 20 mole% ratio making inactivation of all the catalyst by trace amounts of sulphurous compounds improbable.

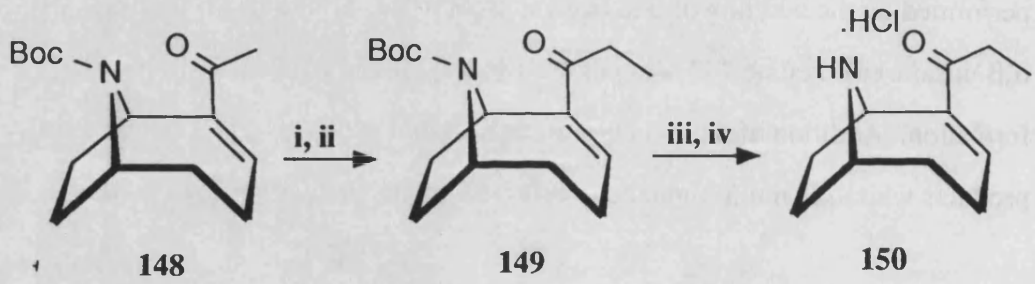
An alternative hypothesis is that when in the vicinity of the aryl halide the palladium becomes coordinated by the tertiary bicyclic amine and is therefore not available to enter into a catalytic carbonylation cycle. A similar observation has been made by Stille in the palladium catalysed carbonylation of an aryl triflate containing a primary amine.[122]

The possibility of forming an aryl ester by reaction of the aryllithium compound, obtained by halogen-metal exchange, and reaction with a chloroformate reagent was investigated. Halogen-metal exchange with aryl iodide **142** was performed by the addition of one equivalent of n-butyllithium. The corresponding α,β-unsaturated ketone **143** was not used so as to avoid competitive enolate formation. Addition of methyl chloroformate gave a low yield of a mixture of products which ^{1}H n.m.r. indicated contained only a small amount of methyl ester.

This result may be due to the aryllithium reacting with n-iodobutane, formed in the halogen-metal exchange reaction, to give the n-butyl substituted product. This reaction is known to be strongly promoted when THF is used as solvent.[123] However, the complex nature of the reaction mixture prevented isolation and identification of the n-butyl substituted product if it was indeed present.

2.7. Alkylation of the kinetic enolate of *N*-Boc anatoxin.

We envisaged that instead of producing a C-11 functionalised derivative of anatoxin by alkylation of the allylic anion obtained from ketene-S,S-acetal **77** the same objective could be accomplished by alkylation of the kinetic enolate of the



148 149 150

Figure 69 : *Reagents and conditions :* i, LDA, THF, 0°C, 1h; ii, MeI, THF, 0°C; iii, CF_3CO_2H, 0°C, 15min; iv, HCl, Et_2O.

α,β-unsaturated methyl ketone group to give the homologated ketone. We used *N*-Boc protected anatoxin as this compound was easier to handle and analyse by t.l.c. than the free amine and also it should prevent any unwanted side reactions arising from amine deprotonation.

N-Boc anatoxin **148** was obtained by protection of the secondary amine group in anatoxin, prepared by the method of Lindgren *et al.*,[45] followed by protection of the secondary amine group in anatoxin with di-*t*-butyldicarbonate. The kinetic lithium enolate was formed by the addition of LDA at 0°C to *N*-Boc anatoxin (figure 69). Trapping with iodomethane gave the ethyl ketone **149** in 41% yield. Removal of the Boc group was accomplished with trifluoroacetic acid and the secondary amine isolated as the hydrochloride salt **150** in 63% yield. These reactions have only been performed on a small scale and the yields are as yet unoptimised.

This may provide a route to C-11 functionalised derivatives of anatoxin which contain nitrile or ester groups in the spacer-arm for use in immobilising the affinity ligand. Since nitriles and esters are typically 5 pK_a units less acidic than ketones,[90] it should therefore be possible to use these functional groups in the presence of a ketone enolate without enolate equilibration occurring.

Another possibility which arises with ethyl ketone **150** is the potential to form a radio-labelled probe for the nAChR. This is dependent on the ethyl ketone displaying binding affinity and selectivity comparable with that of anatoxin. The radio-labelled probe would be available by alkylation of the kinetic enolate of methyl ketone **148** with iodo tritritiomethane (CT_3I). The radioactive nuclei included in this way would be non-exchangable and so activity would not be lost during removal of the Boc group with trifluoroacetic acid other than through decay of the radioactive nuclei. The removal of the Boc group is a rapid, one step process

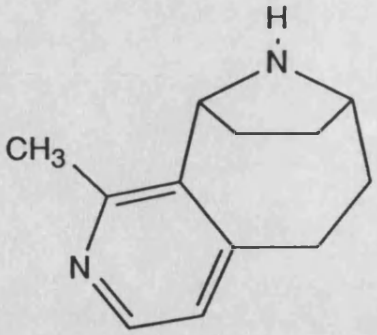
and so a radio-labelled ligand with a high degree of incorporated radioactivity should be available.

2.8. Suggestions for future work.

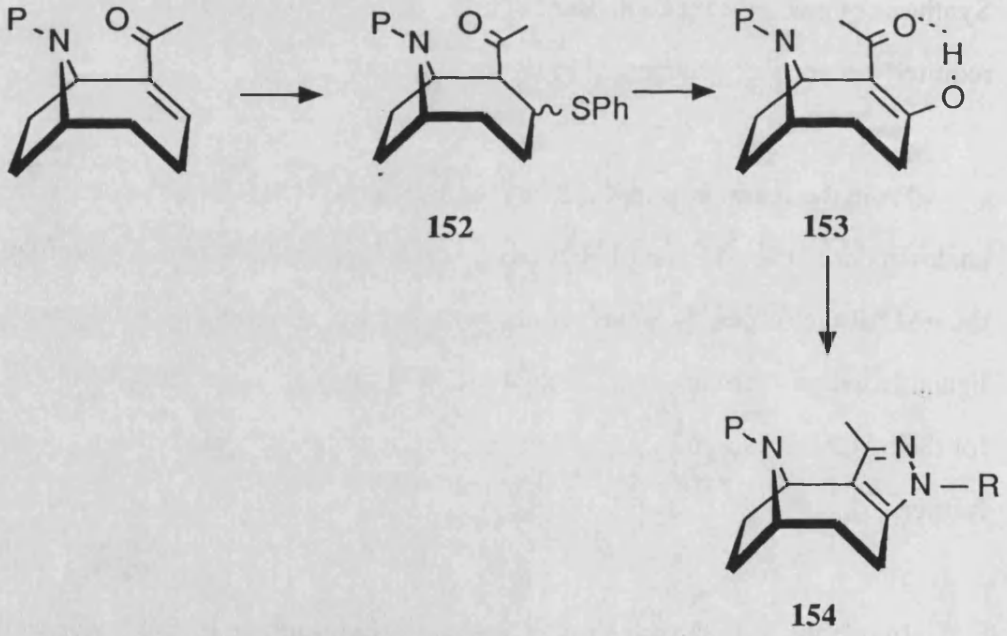
It seems a reasonable assumption that both the amine and the α,β-unsaturated ketone groups of anatoxin are intimately involved in activity and recognition at the receptor site. Any factors which change the steric or electronic environment of these groups may have a detrimental effect on the binding affinity and specificity compared with anatoxin. For an effective affinity ligand we therefore need to be able to functionalise anatoxin with a minimum effect on these two groups. Synthesis of anatoxin functionalised at positions other than C-11 is therefore required *e.g.* the work suggested in figure 29.

From the initial affinity binding studies it appears that both the two substituted anatoxin derivatives **129** and **150** retain a high degree of affinity and specificity for the nAChR (see appendix). Therefore, at the present it seems likely that an affinity ligand based on anatoxin substituted at the C-11 position and a radio-labelled probe for the nAChR could be formed. These lead compounds should be investigated further.

In relation to this work a molecular modelling study of anatoxin derivatives would be informative. A study of the effect of introducing substituents at different positions of anatoxin on the ring conformations and relative orientation and spacing of the amine and α,β-unsaturated ketone should be made. This should indicate where to functionalise anatoxin to cause the minimum of conformational



151



152 153 154

P = Protecting group

Figure 70 : Possible route to fused heterocycles, based on anatoxin, via Pummerer rearrangement to 1,3-diketone.

rearrangement between anatoxin and the substituted derivative.

Another area of synthetic interest is the formation of fused heterocyclic systems based on anatoxin. By analogy with nicotine it may be that these systems would retain binding affinity and specificity. A synthesis of a hybrid anatoxin-nicotine structure **151** has been reported.[124] The compound was reported to bind to cholinergic receptors but approximately 100 times less strongly than anatoxin. A possible route to other fused heterocyclic systems is shown in figure 70. Starting from a suitably protected form of anatoxin, conjugate addition of phenyl sulphide should afford β-keto sulphide **152**. Oxidation of sulphur followed by Pummerer rearrangement should afford the 1,3-diketone **153** from which a range of heterocycles should be available *e.g.* by reaction with hydrazine to form a 1,2-diazole **154**.

The deprotected form of 1,3-diketone **153** will be of interest in itself as the methyl ketone will be locked in one conformation by an internal hydrogen bond. A decision about whether it is the s-*cis* or s-*trans* α,β-unsaturated ketone conformation which is involved in binding may then be possible by carrying out a binding affinity assay on this compound.

3. Appendix

Competition binding assays were performed by Dr. S. Wonnacott, Department of Biochemistry, University of Bath. Assays were performed with a semi-crude rat brain P2 membrane fraction. Whole brains from male Wistar rats (minus the cerebellum) were homogenised (10% w/v) in 0.32M sucrose solution, pH 7.4, containing 1mM EDTA, 0.1mM phenylmethylsulphonyl fluoride and 0.01% (w/v) sodium azide and the suspension centrifuged at 1000 × *g* for 10min. The supernatant was decanted and retained on ice. The pellet was resuspended in 0.32M sucrose solution (5ml/g original wet weight) and centrifuged. The supernatants were combined and centrifuged at 12,000 × *g* for 30min to give a P2 pellet. This was resuspended in 50mM potassium phosphate buffer, pH 7.4, containing protease inhibitors as above, to give a final volume of 2.5ml/g original wet weight and washed twice by centrifugation and resuspension.

The membrane suspension was diluted 5-fold with 50mM potassium phosphate buffer, pH 7.4 and then preincubated with serial dilutions of drug for 10min at 20°C. 10nM (-)-[^{3}H]Nicotine was incubated for 30min at 20°C in the presence and absence of unlabelled nicotine (10^{-3}M) to determine nonspecific binding. The samples were chilled on ice, diluted with 2ml ice-cold phosphate buffered saline (10mM potassium phosphate, pH 7.4, containing 140mM sodium chloride) and rapidly filtered under vacuum on Whatman GFC filters presoaked in 0.3% (w/v) polyethyleneimine. The filters were washed twice with 2ml phosphate buffered saline; filtration and washing was accomplished within 20s. The filters were counted in 5ml Optiphase "safe" scintillant in a Packard scintillation spectrometer.

Using this method it was found that *N*-methyl anatoxin **95** exhibited a greater affinity for the nAChR in these membrane preparations than did anatoxin. However, it was also found that this ligand was less specific than anatoxin in its binding.

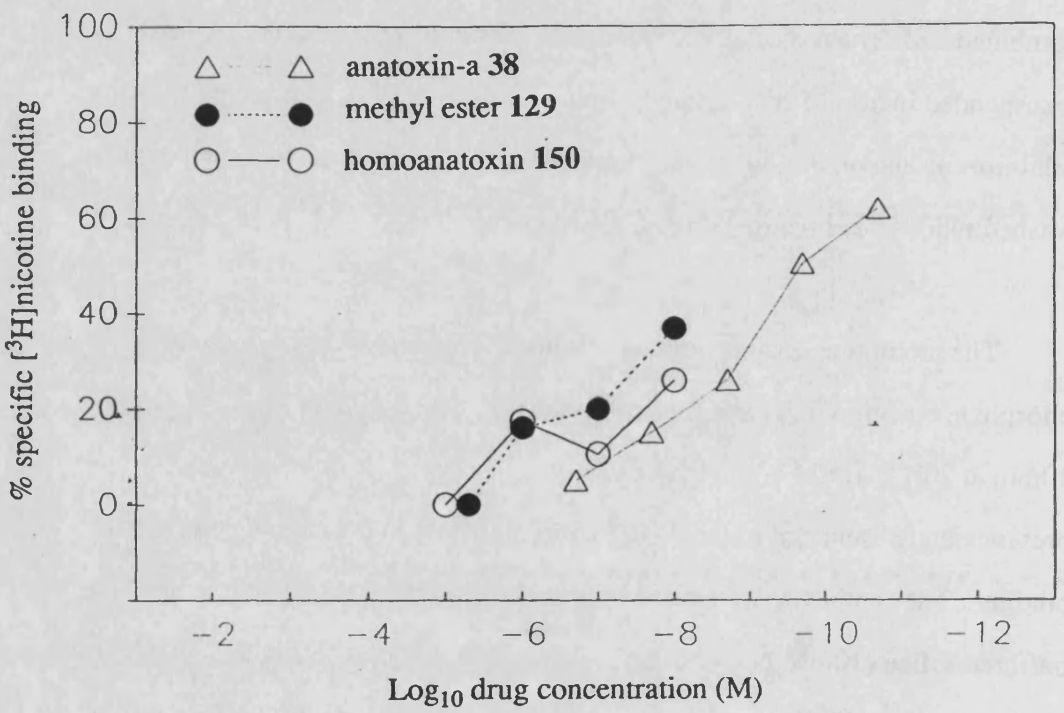


Figure 71 : Competition binding studies.
Anatoxin derivatives versus [^{3}H]nicotine binding to rat brain membranes.

Therefore, it seemed that it was necessary to use anatoxin derivatives in which the nitrogen was present as a secondary amine (rather than a tertiary methylamine) so that specificity for the nAChR was retained.

It was found with benzyl ketone **140** that little or no competitive binding for the nAChR was occurring. This was observed despite the nonspecific activating effect of the tertiary methylamine group. Therefore it appears that a phenyl substituent cannot be accommodate at C-11 whilst retaining binding activity.

The C-11 alkyl substituted derivative **129**, with a terminal methyl ester group, when assayed was found to bind to the nAChR but to a lesser extent than anatoxin itself (figure 71). However, the binding affinity is still sufficiently high that an effective affinity ligand may be preparable from this compound.

Homoanatoxin-a **150** exhibited a binding affinity which was intermediate between that of the methyl ester **129** and anatoxin (figure 71). It appears therefore that the tritium labelled derivative of this compound may well be of use in the identification and investigation of the nAChR in rat brain membranes and other brain preparations.

Whilst the binding affinities of the above compounds have been studied no attempt has been made to measure the efficacy of these compounds, that is their activity at the binding site. Similarly, no attempt has been made to form the above compounds in optically pure form and measure the binding affinity of each individual isomer. One optical pure isomer of compounds **129** and **150** may exhibit increased binding affinity compared with the racemate of these compounds whilst the opposite optical isomer may exhibit decreased binding or no binding for the nAChR.

In a separate study Professor R. J. Walker of The School of Biochemical and Physiological Sciences, University of Southampton, has studied the effects of racemic anatoxin (prepared in our laboratories) on the AChR on the muscle cells of the nematode *Ascaris*. Anatoxin was found to be cholinomimetic in *Ascaris* but less potent than acetylcholine. A relative potency of 0.17±0.04 was measured. These results indicate the usefulness of anatoxin in the identification and characterisation of acetylcholine receptors from various sources.

4. Experimental

Instrumentation and Experimental Techniques

Infrared spectra were recorded in the range 4000-600 cm^{-1} using a Perkin-Elmer 1310 grating spectrophotometer and peaks are reported (υ_{max}) in wavenumbers (cm^{-1}) with reference to the polystyrene 1601 cm^{-1} peak. The abbreviation "br" is appended to a peak to indicate significant broadening. Spectra of liquid samples were taken as thin films, or as solutions in chloroform ($CHCl_3$). Spectra of solid samples were taken by precipitation of a thin film of sample, from a suitable volatile solvent, on to a sodium chloride disc or as solutions in chloroform.

Routine mass spectra from electron ionisation (E.I.), chemical ionisation (C.I.) and high resolution accurate mass determination were recorded with a VG Analytical 7070E instrument with a VG2000 data system. Unless otherwise stated the data provided is that from electron ionisation with an ionising potential of 70eV. Chemical ionisation was performed with *iso*-butane as reagent gas. Where possible, the molecular ion peak (M^+) and base peak are indicated, as are all sizeable fragments with assignments.

Proton magnetic resonance (^1H n.m.r.) spectra were recorded at 60MHz on Hitachi Perkin-Elmer high resolution R-23B and Varian Anaspect EM-360 spectrometers and at 270MHz on a Jeol GNM GX FT 270 spectrometer. Carbon 13 magnetic resonance (^{13}C n.m.r.) spectra were recorded on a Jeol GNM GX FT 270 spectrometer operating at 67.8MHz and using 90 and 135 DEPT pulse sequences to aid in spectral assignment. ^1H and ^{13}C n.m.r. spectra were recorded in deuterochloroform ($CDCl_3$) and are expressed in parts per million (δ) downfield from internal tetramethylsilane. Multiplicities are given as follows: singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m). The abbreviation "br" is appended to a multiplicity to indicate significant broadening.

Melting points (m.p.) were determined on commercially available apparatus (Gallenkamp) and are uncorrected. Elemental microanalyses were carried out using a Carlo Erba 1106 Elemental Analyser. Optical rotations were measured using a Perkin-Elmer 141 polarimeter.

Thin layer chromatography (t.l.c.) was used extensively as a qualitative guide during reactions and for assessing the purity of compounds. Merck DC-alufolien Kieselgel 60 F_{254} and Whatman AL SIL G/UV sheets containing fluorescent indicator were used for this purpose. Visualisation of reaction components was achieved by illumination under short wavelength (254 nm) ultraviolet light (when possible) or using a reagent which would give a colour change with the functional groups present, as described in "Dyeing Reagents for Thin Layer and Paper Chromatography", E. Merck, Darmstadt, 1980.

Unless otherwise stated petroleum refers to that fraction of petroleum spirit boiling in the range 60-80°C. Solvents used as eluants in chromatography were dried and distilled prior to use except for diethyl ether which was dried over sodium wire and used without distillation.

Medium pressure flash column chromatography was routinely employed using Kieselgel 60 (Merck 9385) and 60H silica gel (Merck 7736) for reaction component seperations. A pressure gradient was developed using small, commercially available hand bellows (Gallenkamp). In all cases columns were prepared in the least polar solvent of the eluant mixture and chromatography was carried out with the least polar solvent as initial eluant, then eluting with solvent mixtures of steadily increasing polarity. Material to be chromatographed was pre-adsorbed onto the column support and applied as a thin layer to the top of the column.

In those cases where reduced pressure distillation was difficult, if not destructive to the higher boiling compounds, or when column chromatography was particularly difficult, samples were purified employing preparative, centrifugally accelerated, thin-layer radial chromatography (Model 7924 Chromatotron). Adsorbent layers (2 mm thickness of silica gel PF_{254} type 60 TLC from Merck (7749)) coated on circular glass plates were used for large sample loadings of up to 300 mg total sample.

Tetrahydrofuran (THF) was pre-dried over sodium wire, then refluxed over sodium benzophenone ketyl under dry nitrogen until anhydrous. This was redistilled immediately prior to use.

Glassware used for water sensitive reactions was baked in an oven at 120°C for approximately 12h and allowed to cool in a desiccator over $CaCl_2$. Flasks and stirrer bars were, however, additionally flame dried under a stream of dry nitrogen.

In all experiments the excess solvent was removed with a Buchi rotary evaporator using a water aspirator at room temperature to avoid unnecessary decomposition. All yields quoted are of purified products and are uncorrected unless otherwise stated.

All other reagents and solvents were purified and dried when required using the methods described in D.D. Perrin, W.L.F. Armarego and D.R. Perrin, "Purification of Laboratory Chemicals", 2nd Edn., Pergamon Press, Oxford, 1980.

9-((4-Methylbenzene)sulphonyl)-9-azabicyclo[4.2.1]nonan-2-one (**45**)

To a solution of vinylcarbamate (**97**) (494 mg, 2.36 mmol) in *p*-dioxane (20 ml) was added water (5 ml) $HCl_{(aq)}$ (S.G. 1.16) (0.5 ml, ≈5.1 mmol) and the mixture heated to reflux under nitrogen for 7h. The reaction mixture was cooled in ice-water and basified with 20M $NaOH_{(aq)}$. The *p*-dioxane was removed *in vacuo*, the residue poured into water (10 ml) and extracted with methylene chloride (5 x 5 ml). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo*. The crude amine was dissolved in pyridine (10 ml) at 0°C and tosyl chloride (591 mg, 3.10 mmol) added. After 16h the majority of the pyridine was removed *in vacuo*, the residue poured into 2M $HCl_{(aq)}$ (10 ml) and extracted with methylene chloride (5 x 5 ml). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the sulphonamide (**45**) as a colourless solid which could be purified further by recrystallisation from methanol (404 mg, 58%), m.p. 155°C (methanol); R_F (ethyl acetate-petroleum 2:3) = 0.55. Spectral and analytical data as previously reported.[54]

(R)-α-Ethyl *N*-((4-methylbenzene)sulphonyl)-glutamate ((-)-**52**)

To a solution of enzymatically resolved allene ((-)-**41**) (206 mg, 0.637 mmol) in ethyl acetate (10 ml) at -78°C was added an excess of ozone in oxygen to form a blue/purple coloured solution. After 2.5 hours 30% $H_2O_{2(aq)}$ (10 ml, ≈120 mmol) was added and the mixture heated to reflux for 2.5 hours. The reaction mixture was extracted with 1M $NaOH_{(aq)}$ (4 x 10 ml), the extracts acidified with $HCl_{(aq)}$, extracted with ethyl acetate (4 x 10 ml), the extracts dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the carboxylic acid ((-)-**52**) as a clear pale yellow oil (119 mg, 57%); R_F (ethyl acetate-petroleum 4:1) = 0.49; $[\alpha]^{20}_D$ = -31.8° (c = 1.2, $CHCl_3$); Spectral data as previously reported.[60]

cis-5-(2-Bromoethyl)-1-((4-methylbenzene)sulphonyl)-2-(2-(phenylsulphinyl)-ethanon-1-yl)-pyrrolidine (55)

To a solution of ester (54) (170 mg, 0.42 mmol) in THF (5 ml) at -78°C was added lithiomethyl phenyl sulphoxide (0.84 mmol) [formed by addition of Bu^nLi (1 equiv.) to methyl phenyl sulphoxide (1 equiv.) in THF] in THF (4 ml). Saturated $NH_4Cl_{(aq)}$ (2 ml) was added after 20min, the reaction mixture poured into water (20 ml) and extracted with methylene chloride (5 x 10 ml). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the β-ketosulphoxide (55) as a mixture of diastereomers (159 mg, 76%); R_F (ethyl acetate-petroleum 7:3) = 0.53 and 0.45; υ_{max} ($CHCl_3$) 2970 (alkane C-H), 1720 (C=O), 1675, 1600, 1440, 1350 (-SO_2-), 1310, 1160 (-SO_2-), 1090, 1035br (S=O) and 1000 cm^{-1}; δ_H (270 MHz) 1.30-2.60 (7H, m, 3 C*H*$_2$ and C5*H*), 2.45 2.46 (3H, s, Ar-C*H*$_3$), 3.30-3.46 (1H, m), 3.50-3.64 (1H, m), 3.72-3.91 (1H, m), 3.98-4.14 (1H, m), 4.20-4.40 (1H, m) and 7.32-7.78 (9H, m, aromatic); m/z (E.I.) 332 ($M(^{81}Br)^+$-$C_6H_5SOCH_2CO$, 30%), 330 ($M(^{79}Br)^+$-$C_6H_5SOCH_2CO$, 30), 155 ($ArSO_2^+$, 40), 91 ($C_7H_7^+$, 83) and 41 (100); m/z (C.I.) 500 ($M(^{81}Br)H^+$, 2%), 498 ($M(^{79}Br)H^+$, 2), 484 ($M(^{81}Br)H^+$- O, 12) 482 ($M(^{79}Br)H^+$-O, 11) and 111 (100).

9-((4-Methylbenzene)sulphonyl)-3-phenylsulphinyl-9-azabicyclo[4.2.1] nonan-2-one (56)

To a solution of bromide (55) (167 mg, 0.335 mmol) in dry DMSO (5 ml) was added NaH (17.2 mg, 0.717 mmol) and the mixture stirred at room temperature under nitrogen for 2 h. Saturated $NH_4Cl_{(aq)}$ (2ml) was added, the mixture poured into water (15 ml) and extracted with methylene chloride (5 x 10 ml). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the bicyclic β-ketosulphoxide (56) as a mixture of diastereomers (61 mg, 44%); R_F (ethyl acetate-petroleum 7:3) = 0.30; δ_H (270 MHz) 1.10-2.30 (8H, m, 4 C*H*$_2$), 2.44 (3H, s, Ar-C*H*$_3$), 4.04-4.68 (3H, m, C1*H*, C6*H* and COC*H*SO) and 7.27-7.90 (9H,

m, aromatic).

9-((4-Methylbenzene)sulphonyl)-9-azabicyclo[4.2.1]non-3-en-2-one (57)

A solution of bicyclic β-ketosulphoxide (**56**) (259 mg, 0.62 mmol) in toluene (10 ml) was heated to reflux for 3h. The toluene was removed *in vacuo* and the residue purified by chromatography followed by recrystallisation from methanol to give the bicyclic α,β-unsaturated ketone (**57**) as off-white needles (87 mg, 48%), m.p. 201.6-201.8°C (methanol); (Found : C, 61.8; H, 5.95; N, 4.69. $C_{15}H_{17}NO_3S$ requires C, 61.83; H, 5.88; N, 4.81%); R_F (ethyl acetate-petroleum 3:2) = 0.58; υ_{max} ($CHCl_3$) 2980br (alkane C-H) 1665 (α,β-unsaturated ketone), 1600, 1400, 1350 (-SO_2-), 1290, 1160 (-SO_2-), 1100, 1075, 1045, 995, 935 and 915 cm^{-1}; δ_H (270 MHz) 1.58-2.20 (4H, m, 2 C*H*$_2$), 2.43 (3H, s, Ar-C*H*$_3$), 2.50 (1H, dm, J 20Hz, C5*H*), 3.06 (1H, dm, J 20Hz, C5*H*), 4.46-4.59 (2H, m, C1*H* and C6*H*), 5.87 (1H, dm, J 13Hz, C3*H*), 6.22 (1H, dm, J 13Hz, C3*H*) and 7.26-7.75 (4H, m, aromatic); m/z (E.I.) 136 (M^+-$ArSO_2$, 100%); m/z (C.I.) 292 (MH^+, 100%) and 136 (MH^+-$ArSO_2H$, 57).

9-((4-Methylbenzene)sulphonyl)-9-azabicyclo[4.2.1]non-4-en-2-one (63)

A solution of bicyclic α,β-unsaturated ketone (**57**) (11.3 mg, 0.039 mmol) in DME (1 ml) was added to a solution of lithio methoxymethyldiphenylphosphine oxide [formed by the addition of LDA (0.096 mmol) in DME (1 ml) to a solution of methoxymethyldiphenylphosphine oxide (23 mg, 0.093 mmol) in DME (1 ml) at -78°C] at -78°C under argon. After 20 min saturated $NH_4Cl_{(aq)}$ (1 ml) was added, the reaction mixture poured into water (10 ml) and extracted with methylene chloride (4 x 5 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave reclaimed α,β-unsaturated ketone (**57**) (5.9 mg, 52%) and the β,γ-unsaturated bicyclic ketone (**63**) (3.4 mg, 30%); R_F (ethyl acetate-petroleum 3:2) = 0.74; υ_{max} ($CHCl_3$) 2930 (alkane C-H), 1720 (C=O), 1600, 1355 (-SO_2-), 1165 (-SO_2-), 1100, 1035, 1020, 980 and 935 cm^{-1}; δ_H (270 MHz)

1.20-2.30 (4H, m, 2 C*H*$_2$), 2.44 (3H, s, Ar-C*H*$_3$), 2.77 (1H, dd, J 15.7 and 8.8Hz, C3*H*), 3.96 (1H, dm, J 16Hz, C3*H*), 4.45 (1H, dd, J 10.4 and 3.9Hz, C6*H*), 4.81-4.90 (1H, m, C1*H*),5.39 (1H, ddm, J 11.3 and 8.8Hz, C4*H*),5.59 (1H, dm, J 11.3Hz, C5*H*) and 7.28-7.76 (4H, m, aromatic); m/z (E.I.) 263 (M^+-CO, 74%), 155 (Ts^+, 16), 136 (M^+-Ts, 20), 108 (M^+-(CO and Ts), 100) and 91 ($C_7H_7^+$, 88); m/z (C.I.) 292 (MH^+, 100%).

(10R)-9-(1-Phenylethyl)-9-azabicyclo[3.3.1]nonan-1-ol (**69**)

To a solution of the bicyclic hemiketal (**65**) (1 g, 7.03 mmol) in degassed water (7 ml) was added (R)-α-methylbenzylamine (1.1 ml, 8.5 mmol) and *p*-toluenesulphonic acid monohydrate (0.14 g, 0.74 mmol). The rapidly stirred emulsion was heated to 100°C under nitrogen in a sealed tube for 7 days. The reaction mixture was extracted with ether (5 x 5 ml), the combined extracts washed with brine (10 ml) and the brine back-extracted with ether (2 x 5 ml). The combined ether layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the bicyclic hemi-aminal (**69**) as a pale brown mobile oil (0.36 g, 21%); R_F(ethyl acetate-petroleum 1:4) = 0.58; (Found: M^+, 245.1790. $C_{16}H_{23}NO$ requires M^+, 245.1778); $[\alpha]^{20}_D = 40.5°$ (c = 3.1, $CHCl_3$); υ_{max} (thin film) 3700-3150 (O-H), 3080 (aryl C-H), 3040 (aryl C-H), 2940br (alkane C-H), 1690 (aromatic), 1605, 1450, 1365, 1270, 1210, 1170, 1155, 1030, 995, 765 and 705 cm^{-1}; δ_H (270 MHz) 1.20-2.30 (13H, m, 6 C*H*$_2$ and O*H*), 1.32 (3H, d, J 6.8Hz, CH-C*H*$_3$),4.21 (1H, m, C5*H*), 4.37 (1H, q, J 6.8Hz, CH_3-C*H*) and 7.10-7.44 (5H, m, aromtic); δ_C (67.8 MHz) 20.1 (*C*H$_2$), 20.4 (*C*H$_2$), 27.3 (*C*H$_3$), 28.3 (*C*H$_2$), 28.8 (*C*H$_2$), 32.9 (*C*H$_2$), 37.3 (*C*H$_2$), 49.6 (*C*5), 70.2 (*C*10), 82.7 (*C*1), 125.9 (aromatic), 126.2 (aromatic), 128.0 (aromatic) and 149.2 (aromatic); m/z (E.I.) 245 (M^+, 45%) and 105 ($PhCHCH_3^+$, 100).

(10R)-9-(1-Phenylethyl)-9-azabicyclo[4.2.1]nonan-2-one (**70** and **71**)

A solution of hemi-aminal (**69**) (307 mg, 1.25 mmol) in glacial acetic acid was heated to 90°C for 3 hours under nitrogen. Pyridinium hydrobromide perbromide (406 mg, 1.27 mmol) was added and heating at 90°C continued for 4 hours before heating to reflux for 16 hours. The glacial acetic acid was removed *in vacuo*, the residue taken up in water (10 ml) and basified with $K_2CO_{3(s)}$. The basic solution was extracted with methylene chloride (5 x 5 ml), the combined organic layers dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the bicyclic ketones (**70** and **71**) as an inseparable mixture of diastereomers (69 mg, 23%); $[\alpha]^{20}_D = 36.6°$ (c = 0.7, $CHCl_3$); υ_{max} (thin film) 3070 (aryl C-H), 3040 (aryl C-H), 2930br (alkane C-H), 1685br (C=O), 1600 (aromatic), 1495, 1410, 1360, 1320, 1260, 1155, 1115, 1085, 1025, 915, 835, 765 and 705 cm^{-1}; δ_H (270 MHz) 1.34 (6H, d, J 6.5Hz, 2 CH-C*H*$_3$), 1.40-3.86 (25H, m, 5 C*H* and 10 C*H*$_2$), 3.94 (1H, q, J 6.5Hz, CH_3-C*H*) and 7.18-7.40 (10H, m, aromatic); m/z (E.I.) 215 (M^+-CO, 46%) and 105 ($PhCHCH_3^+$, 100); m/z (C.I.) 244 (MH^+, 79%), 215 (MH^+-HCO, 100) and 105 ($PhCHCH_3^+$, 96).

9-Methyl-2-(2-methyl-1,3-dithian-2-yl)-9-azabicyclo[4.2.1]non-2-ene (**78**)

To a solution of ketene-*S*,*S*-acetal (**77**) (1 g, 3.91 mmol) in THF (10 ml) at -78°C was added 1.6M Bu^nLi in hexane (2.95 ml, 4.72 mmol). The mixture was warmed to room temperature and left to stir for 3 hours before cooling to -78°C and adding MeI (557 mg, 3.92 mmol) in THF (2.2 ml). After 15 min saturated $NH_4Cl_{(aq)}$ (5 ml) was added. The reaction mixture was poured into water (20 ml), extracted with methylene chloride (5 x 10 ml), the combined extracts dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the substituted 2-methyl-1,3-dithiane (**78**) as a colourless solid (773 mg, 73%), m.p. 72.5°C (petroleum) (lit.,[45] 69-72°C, *iso*-propyl ether). R_F (diethyl ether-methanol 3:1) = 0.12. Spectral data as previously reported.[45]

N-Methyl anatoxin hydrochloride (**95**)

To a solution of substituted 2-methyl-1,3-dithiane (**78**) (25 mg, 0.093 mmol) in *p*-dioxane (2.3 ml) was added water (0.7 ml) and $HCl_{(aq)}$ (S.G. 1.18) (0.1 ml, ≈1.2 mmol). The solution was heated to reflux under nitrogen for 4 hours before cooling in ice/water and basifying with 20M $NaOH_{(aq)}$. The *p*-dioxane was removed *in vacuo*, the residue poured into water (5 ml) and extracted with methylene chloride (5 x 3 ml). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo*. The residue was treated with ethereal hydrogen chloride before chromatography to give *N*-methyl anatoxin hydrochloride (**95**) as a colourless glass (8.5 mg, 42%); R_F (methylene chloride-methanol 9:1) = 0.33. Spectral data as previously reported.[46g]

2-(1,3-Dithian-2-yl)-9-methyl-9-azabicyclo[4.2.1]non-2-ene (**96**)

The anion of ketene-*S*,*S*-acetal (**77**) was formed (as described for compound **78**) using ketene-*S*,*S*-acetal **77** (101 mg, 0.395 mmol) and 1.6M Bu^nLi in hexane (0.29 ml, 0.46 mmol). The solution of anion was cooled to -78°C and saturated $NH_4Cl_{(aq)}$ (1 ml) added. Extraction and purification as described above (compound **78**) gave monosubstituted-1,3-dithiane (**96**) as a pale yellow oil (76 mg, 75%); R_F (ethyl acetate-petroleum 9:1) = 0.19; (Found m/z 255.1116. $C_{13}H_{21}NS_2$ requires m/z 255.1114); υ_{max} (thin film) 2920br (alkane C-H), 1650 (C=C), 1465, 1445, 1430, 1420, 1305, 1275, 1250, 1210, 1165, 1020, 910, 835, 765, 735 and 680 cm^{-1}; δ_H (270 MHz) 1.15-2.50 (10H, m, 5 C*H*$_2$), 2.42 (3H, s, N-C*H*$_3$), 2.70-3.08 (4H, m, 2 S-C*H*$_2$), 3.40-3.50 (1H, m, C6*H*), 3.76 (1H, dbr, J 8.8Hz, C1*H*), 4.54 (1H, s, S-C*H*-S) and 5.92 (1H, dbr, J 7.7Hz, C3*H*); m/z (E.I.) 255 (M^+, 100%), 240 (M^+-CH_3, 4) and 136 (M^+-$C_4H_7S_2$, 31).

9-Vinyloxycarbonyl-9-azabicyclo[4.2.1]nonan-2-one (97)

To a solution of tertiary amine (**68**) (96 mg, 0.626 mmol) in methylene chloride (3 ml) was added anhydrous $K_2CO_{3(s)}$ (≈50 mg, 0.36 mmol), vinyl chloroformate (59 µl, 0.69 mmol) and the mixture heated to reflux in the dark, under nitrogen for 4h. The reaction mixture was concentrated *in vacuo*. Chromatography gave the vinyl carbamate (**97**) as a clear colourless oil (88mg, 67%); R_F (ethyl acetate-petroleum 1:4) = 0.32; υ_{max} (thin film) 3125 (alkene C-H), 2990br (alkane C-H), 1720br (ketone and carbamate C=O), 1650, 1420, 1380, 1345, 1245, 1200, 1160, 1100, 1020, 990, 960, 880, 840 and 770 cm^{-1}; δ_H (270 MHz) 1.50-2.85 (10H, m, 5 CH_2), 4.40-4.54 (1H, m, C6*H*), 4.46 (0.6H, dd, J 6.2 and 1.6Hz, 0.6 OC=CH_{trans}), 4.51 (0.4H, dd, J 6.2 and 1.6Hz, 0.4 OC=CH_{trans}), 4.58-4.68 (1H, m, C1*H*), 4.76 (0.6H, dd, J 13.8 and 1.6Hz, 0.6 OC=CH_{cis}), 4.85 (0.4H, dd, J 13.8 and 1.6Hz, 0.4 OC=CH_{cis}), 7.21 (0.6H, dd, J 13.8 and 6.2Hz, 0.6 OC*H*=C) and 7.26 (0.4H, dd, J 13.8 and 6.2Hz, 0.4 OC*H*=C); m/z (E.I.) 209 (M^+, 16%), 166 (M^+-C_2H_3O, 15), 138 (M^+-$C_3H_3O_2$, 79) and 95 ($C_5H_5N^+$,100).

2-(1,3-Dithian-2-ylidene)-9-(2-methylpropan-2-yl)-oxycarbonyl-9-azabicyclo [4.2.1]nonane (98)

To a solution of vinylcarbamate (**97**) (101 mg, 0.482 mmol) in *p*-dioxane (4 ml) was added water (1 ml) and $HCl_{(aq)}$ (S.G. 1.16) (0.5 ml, ≈5.1 mmol). The mixture was heated to reflux under nitrogen for 7h. The reaction mixture was cooled in ice-water and basified with 20M $NaOH_{(aq)}$. The *p*-dioxane was removed *in vacuo*, the residue poured into water (5 ml) and extracted with methylene chloride (5 x 2 ml). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo*. The crude amine was dissolved in THF (3 ml) and 2-lithio-2-trimethylsilyl-1,3-dithiane (1.2 equiv.) in THF (2 ml) added at -78°C under nitrogen. After 1h saturated $NH_4Cl_{(aq)}$ (1 ml) was added, the reaction mixture poured into water (5 ml) and extracted with methylene chloride (5 x 2 ml). The combined extracts were concentrated *in vacuo*

and the residue taken up in 2:1 THF-water (3 ml). Triethylamine (166 µl, 1.19 mmol) and di-*t*-butyldicarbonate (389 mg, 1.78 mmol) were added and the mixture stirred at room temperature for 24h. The THF was removed *in vacuo*, the residue poured into water (5 ml) and extracted with methylene chloride (5 x 2 ml). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the ketene-*S*,*S*-acetal (**98**) as a colourless, viscous oil (72 mg, 44%); R_F (ethyl acetate-petroleum 1:9) = 0.42; (Found m/z 341.1482. $C_{17}H_{27}NO_2S_2$ requires 341.1481); υ_{max} ($CHCl_3$) 2950br (alkane C-H), 1670br (carbamate C=O), 1400, 1370, 1350, 1305, 1165, 1120, 1020, 990, 965, 920 and 870 cm^{-1}; δ_H (270 MHz) 1.00-3.18 (16H, m, 8 C*H*$_2$), 1.43 (4.5H, s, 0.5 C(C*H*$_3$)$_3$), 1.48 (4.5H, s, 0.5 C(C*H*$_3$)$_3$), 4.25-4.45 (1H, m, C6*H*), 5.10-5.23 (1H, m, C1*H*); m/z (E.I.) 341 (M^+, 47%), 285 (M^+-C_4H_8, 74), 240 (M^+-$C_5H_9O_2$, 33) and 57 ($C_4H_9^+$, 100).

9-((2-Methylpropan-2-yl)-oxycarbonyl)-9-azabicyclo[4.2.1]nonan-2-one (**99**)

To a solution of vinylcarbamate (**97**) (60 mg, 0.287 mmol) in *p*-dioxane (2 ml) was added water (0.5 ml) and $HCl_{(aq)}$ (S.G. 1.16) (0.5 ml, ≈5.1 mmol). The mixture was heated to reflux under nitrogen for 3h. The reaction mixture was cooled in ice-water and basified with 20M $NaOH_{(aq)}$. The *p*-dioxane was removed *in vacuo*, the residue poured into water (10ml), extracted with methylene chloride (5 × 1 ml) and the combined extracts concentrated *in vacuo*. The residue was taken up in 2:1 THF-water (3 ml) and triethylamine (53 µl, 0.38 mmol) and di-*t*-butyldicarbonate (228 mg, 1.04 mmol) added. After 24h the THF was removed *in vacuo*, the residue poured into water (10 ml) and extracted with methylene chloride (5 x 4 ml). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the *t*-butyl carbamate (**99**) as a colourless oil (46 mg, 67%); R_F (ethyl acetate-petroleum 1:4) = 0.44; υ_{max} (thin film) 2980br (alkane C-H), 1690br (ketone and carbamate C=O), 1400, 1345, 1250, 1180, 1110, 1020, 990, 960, 935, 890, 870, 835 and 780 cm^{-1}; δ_H (270 MHz) 1.30-2.65 (10H, m, 5 C*H*$_2$), 1.44 (5.9H, s, 0.65

$C(CH_3)_3$), 1.50 (3.1H, s, 0.35 $C(CH_3)_3$) and 4.21-4.58 (2H, m, C1*H* and C6*H*); m/z (E.I.) 155 (M^+-(CO and C_4H_8), 41%) 57 ($C_4H_9^+$, 100); m/z (low eV E.I.) 239 (M^+, 42%), 211 (M^+-CO, 30), 155 (M^+-(CO and C_4H_8), 100) 138 (M^+-Boc, 23).

2-(1,3-Dithian-2-ylidene)-9-((4-methylbenzene)sulphonyl)-9-azabicyclo[4.2.1] nonane (**103**)

To a solution of ketone (**45**) (353 mg, 1.20 mmol) in THF (3 ml) at -78°C was added 2-lithio-2-trimethylsilyl-1,3-dithiane (1.2 equiv.) in THF (10 ml). After 30min saturated $NH_4Cl_{(aq)}$ (2 ml) was added, the mixture poured into water (25 ml) and extracted with methylene chloride (5 x 5 ml). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the ketene-*S*,*S*-acetal (**103**) as a colourless solid which could be further purified by recrystallisation from ethyl acetate-petroleum (292 mg, 61%), m.p. 145-145.5°C (ethyl acetate-petroleum); R_F (ethyl acetate-petroleum 3:7) = 0.67; (Found: C, 57.8; H, 6.41; N, 3.50. $C_{19}H_{25}NO_2S_3$ requires C, 57.7; H, 6.37; N, 3.54); υ_{max} ($CHCl_3$) 2930br (alkane C-H), 1600 (aromatic), 1420, 1340 (-SO_2-), 1305, 1155 (-SO_2-), 1095, 1015, 980 and 915 cm^{-1}; δ_H (270MHz) 1.20-3.15 (16H, m, 8 CH_2), 2.43 (3H, s, Ar-CH_3), 4.35-4.45 (1H, m, C6*H*), 5.29 (1H, dbr, J 9Hz, C1*H*), and 7.26-7.80 (4H, m, aromatic); m/z (E.I.) 395 (M^+, 21%), 240 (M^+-Ts, 100) and 91 ($C_7H_7^+$, 22).

(3-Methyloxetan-3-yl)-methyl 6-bromohexanoate (**108**)

6-Bromohexanoic acid (**107**)(2 g, 10.3 mmol) was warmed until liquid (m.p. 35°C). Thionyl chloride (0.85 ml, 11.7 mmol) was added and the mixture heated to reflux under nitrogen for 0.5h. Unreacted thionyl chloride was removed *in vacuo* and the crude acid chloride redissolved in methylene chloride (5 ml). Pyridine (0.85 ml, 10.5 mmol) and 3-hydroxymethyl-3-methyloxetane (**106**) (1.05 g, 10.3 mmol) were added and the mixture stirred at 0°C for 45min. The reaction mixture was poured into water (25 ml), the organic layer separated and the aqueous layer extracted with

methylene chloride (3 x 5 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the ester (**108**) as a clear pale yellow oil (2.47 g, 86%); R_F (ethyl acetate-petroleum 1:4) = 0.37; υ_{max} (thin film) 2945br (alkane C-H), 2880 (alkane C-H), 1730 (ester C=O), 1455, 1385, 1360, 1250, 1175, 1130, 985, 945, 840, 740 and 645 cm^{-1}; δ_H (60 MHz) 1.10-2.20 (6H, m, 3 CH_2), 1.35 (3H, s, C-CH_3), 2.42 (2H, t, J 7Hz, O_2CCH_2), 3.45 (2H, t, J 7Hz, $BrCH_2$), 4.21 (2H, s, CO_2CH_2) and 4.30-4.75 (4H, m, 2 OCH_2); m/z (E.I.) 179 ($M(^{81}Br)^+$-$C_5H_9O_2$, 48%), 177 ($M(^{79}Br)^+$-$C_5H_9O_2$, 47) and 69 ($C_5H_9^+$, 100); m/z (C.I.) 281 ($M(^{81}Br)H^+$, 98%) and 279 ($M(^{79}Br)H^+$, 100).

4-Methyl-1-(5-bromopentan-1-yl)-2,6,7-trioxabicyclo[2.2.2]octane (**109**)

To a solution of ester (**108**) (546 mg, 1.96 mmol) in dry methylene chloride (3 ml) at 0°C was added freshly distilled boron trifluoride etherate (70 µl, 0.569 mmol) under nitrogen. After 2h triethylamine (0.27 ml, 1.94 mmol) was added, the precipitate of triethylamine-boron trifluoride complex filtered off and the filtrate concentrated *in vacuo*. Chromatography gave the bicyclic ortho ester (**109**) as a clear colourless oil (313 mg, 57%); R_F (ethyl acetate-petroleum 1:4) = 0.55; υ_{max} (thin film) 2930br (alkane C-H), 2870 (alkane C-H), 1460, 1400, 1355, 1335, 1280, 1245, 1195, 1155, 1055, 980, 890, 825, 770, 735, 710 and 645 cm^{-1}; δ_H (60 MHz) 0.80 (3H, s, C-CH_3), 1.10-2.15 (8H, m, 4 CH_2), 3.41 (2H, t, J 7Hz, $BrCH_2$) and 3.90 (6H, s, 3 OCH_2); m/z (E.I.) 250 ($M(^{81}Br)^+$-CH_2O, 14%), 248 ($M(^{79}Br)^+$-CH_2O, 14), 179 ($M(^{81}Br)^+$-$C_5H_9O_2$, 69), 177 (($^{79}Br)^+$-$C_5H_9O_2$, 70) and 69 ($C_5H_9^+$, 100); m/z (C.I) 281 ($M(^{81}Br)H^+$, 93%) and 279 ($M(^{79}Br)H^+$, 100).

(3-Methyloxetan-3-yl)-methyl 6-iodohexanoate (**110**)

To a solution of bromoester (**108**) (997 mg, 3.57 mmol) in acetone (15 ml) was added sodium iodide (2.8 g, 18.7 mmol) and the mixture stirred to room temperature for 1.5h. The reaction mixture was poured into water (100 ml), extracted with ethyl

acetate (5 x 20 ml), the combined organic layers washed with $Na_2S_2O_{3(aq)}$ (20 ml) and the aqueous layer back extracted with ethyl acetate (2 x 10 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the iodoester (**110**) as a clear colourless oil (906 mg, 78%); R_F (ethyl acetate-petroleum 1:4) = 0.37; υ_{max} (thin film) 2945br (alkane C-H), 2880 (alkane C-H), 1735 (ester C=O), 1445, 1430, 1385, 1355, 1250br, 1185br, 1120, 985 and 840 cm^{-1}; δ_H (60MHz) 1.20-2.20 (6H, m, 3 CH_2), 1.35 (3H, s, CH_3), 2.40 (2H, t, J 7Hz, O_2CCH_2), 3.23 (2H, t, J 7Hz, ICH_2), 4.20 (2H, s, CO_2CH_2) and 4.30-4.75 (4H, m, 2 OCH_2); m/z (E.I.) 225 (M^+-$C_5H_9O_2$, 36%), 199 (M^+-HI, 24) and 55 ($C_4H_7^+$, 100); m/z (C.I.) 327 (MH^+, 100%).

1-(5-Iodopentan-1-yl)-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (**111**)

Method 1: To a solution of iodoester (**110**) (1.739 g, 5.33 mmol) in dry methylene chloride (15 ml) at 0°C was added freshly distilled boron trifluoride etherate (0.15 ml, 1.22 mmol). After 2h triethylamine (0.5 ml, 3.59 mmol) was added, the precipitate of triethylamine-boron trifluoride complex filtered off and the filtrate concentrated *in vacuo*. Chromatography gave the bicyclic ortho ester (**111**) as a clear pale yellow oil (1.285 g, 74%); R_F (ethyl acetate-petroleum 1:4) = 0.34; υ_{max} (thin film) 2930br (alkane C-H), 2890 (alkane C-H), 1460, 1400, 1360, 1335, 1275, 1210, 1060br, 995br, 885, 820, 770, 730 and 715 cm^{-1}; δ_H (60MHz) 0.80 (3H, s, C-CH_3), 1.10-2.25 (8H, m, 4 CH_2), 3.22 (2H, t, J 7Hz, ICH_2) and 3.86 (6H, s, 3 OCH_2); m/z (E.I.) 326 (M^+, 4%), 296 (M^+-CH_2O, 55), 225 (M^+-$C_5H_9O_2$, 45), 199 (M^+-HI, 21) and 55 ($C_4H_7^+$, 100).

Method 2: To a solution of bromo-ortho ester (**110**) (256 mg, 0.917 mmol) in acetone (5 ml) was added sodium iodide (0.80 g, 5.34 mmol). After 3h the reaction mixture was poured into water (30 ml) and extracted with ethyl acetate (5 x 5 ml). The combined organic layers were washed with $Na_2S_2O_{3(aq)}$ (10 ml) and the aqueous

layer back-extracted with ethyl acetate (2 x 5 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the bicyclic ortho ester (**111**) as a clear colourless oil (226 mg, 76%); Physical and spectral data identical with material by method 1.

9-Methyl-2-(2-(5-(4-methyl-2,6,7-trioxabicyclo[2.2.2]octan-1-yl)-pentan-1-yl)-1,3-dithian-2-yl)-9-azabicyclo[4.2.1]non-2-ene (**112**)

The anion of ketene-*S*,*S*-acetal **77** was formed (as described for compound **78**) using **77** (200 mg, 0.78 mmol) in THF (5 ml) and 1.6M Bu^nLi in hexane (0.58 ml, 0.93 mmol). The reaction mixture was cooled to -78°C and iodo ortho ester (**111**) (2.66 mg, 0.78 mmol) in THF (3 ml) added. After 30min saturated $NH_4Cl_{(aq)}$ (1 ml) was added, the reaction mixture poured into water (10 ml) and extracted with methylene chloride (5 x 5 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the alkylated 1,3-dithiane (**112**) as a viscous brown/yellow oil (245 mg, 71%); R_F (methanol-diethyl ether 1:4) = 0.28; ν_{max} ($CHCl_3$) 2930br (alkane C-H), 1620 (C=C), 1430br, 1400, 1360, 1220br, 1135, 1090, 1045, 990, 940, 910 and 890 cm^{-1}; δ_H (270MHz) 0.79 (3H, s, C*H*$_3$), 1.15-3.00 (24H, m, 12 C*H*$_2$), 2.42 (3H, s, N-C*H*$_3$), 3.40-3.50 (1H, m, C6*H*), 3.88 (6H, s, 3 OC*H*$_2$), 4.04 (1H, dbr, J 9Hz, C1*H*) and 6.15-6.27 (1H, m, C3*H*); m/z (E.I.) 255 (M^+-$C_{11}H_{18}O_3$, 100%) and 136 (M^+-$C_{15}H_{25}O_3S_2$, 30); m/z (C.I.) 454 (MH^+, 83%) and 256 (MH^+-$C_{11}H_{18}O_3$, 100).

2-(2-(1-Hexen-6-yl)-1,3-dithian-2-yl)-9-methyl-9-azabicyclo[4.2.1]non-2-ene (**113**)

The anion of ketene-*S*,*S*-acetal **77** was formed (as described for compound **78**) using **77** (2.632 g, 10.3 mmol) in THF (20 ml) and 1.6M Bu^nLi in hexane (7.2 ml. 11.5 mmol). 6-Bromohex-1-ene (1.68 g, 10.3 mmol) in THF (10 ml) was added at -78°C. After 30min saturated $NH_4Cl_{(aq)}$ (5 ml) was added, the reaction mixture poured into water (150 ml) and extracted with methylene chloride (5 x 30 ml). The combined

organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the alkylated 1,3-dithiane (**113**) as a clear pale yellow oil (2.930 g, 84%); R_F (diethyl ether-methanol 4:1) = 0.45; (Found: M^+, 337.1844. $C_{19}H_{31}NS_2$ requires M^+, 337.1896); υ_{max} (thin film) 3080 (alkene C-H), 2920br (alkane C-H), 1640 (C=C), 1430br, 1365, 1350, 1310, 1280, 1250, 1220, 1175, 1125, 1100, 995, 910, 840, 785 and 735 cm^{-1}; δ_H (270MHz) 1.20-3.10 (22H, m, 11 C*H*$_2$), 2.39 (3H, s, N-C*H*$_3$), 3.35-3.45 (1H, m, C6*H*), 4.00 (1H, dbr, J 9Hz, C1*H*), 4.88-5.06 (2H, m, C=C*H*$_2$), 5.70-5.88 (1H, m, C=C*H*) and 6.20 (1H, ddd, J 8.3, 3.8 and 2.3Hz, C3*H*); δ_C (67.8MHz) 23.4 (*C*H$_2$), 23.9 (*C*H$_2$), 25.3 (*C*H$_2$), 27.4 (*C*H$_2$), 27.6 (*C*H$_2$), 27.7 (*C*H$_2$), 27.9 (*C*H$_2$), 29.0 (*C*H$_2$), 32.2 (*C*H$_2$), 33.5 (*C*H$_2$), 38.4 (N*C*H$_3$), 39.6, 60.1 (S-*C*-S), 62.7, 64.5, 114.3 (C=*C*H$_2$), 130.1 (*C*3), 138.7 (C=*C*H) and 145.3 (*C*2); m/z (E.I.) 337 (M^+, 43%), 262 (M^+- C_3H_7S, 56), 255 (M^+-C_6H_{10}, 17), 230 (M^+-$C_3H_7S_2$, 57) and 82 ($C_6H_{10}^+$, 100).

2-(2-(1-Hexen-6-yl)-1,3-dithian-2-yl)-9-vinyloxycarbonyl-9-azabicyclo[4.2.1] non-2-ene (**114**)

To a solution of tertiary amine(**113**) (42 mg, 0.124 mmol) in methylene chloride (10 ml) was added vinyl chloroformate (16 µl, 0.188 mmol) and the mixture heated to reflux under nitrogen and in the dark for 20h. The reaction mixture was concentrated to dryness *in vacuo*. Chromatography gave the vinyl carbamate (**114**) as a clear colourless oil (29 mg, 59%); R_F (ethyl acetate-petroleum 3:17) = 0.62; (Found: M^+, 393.1792. $C_{21}H_{31}NO_2S_2$ requires 393.1794); υ_{max} (thin film) 2930br (alkane C-H), 1695 (carbamate C=O), 1645 (C=), 1415, 1360, 1335, 1145, 1090, 1005, 915 and 865 cm^{-1}; δ_H (270MHz) 1.00-3.00 (22H, m, 11 C*H*$_2$), 4.41 (1H, tbr, J 6.1Hz, C6*H*), 4.56-5.15 (5H, m, 2 C=C*H*$_2$ and C1*H*), 5.66-5.87 (1H, m, C-C*H*=C), 6.16-6.32 (1H, m, C3*H*), 7.20 (0.5H, dd, J 14.1 and 6.3Hz, 0.5 OC*H*=C) and 7.21 (0.5H, dd, J 14.1 and 6.3Hz, 0.5 OC*H*=C); m/z (E.I.) 393 (M^+, 100%), 350 (M^+-C_2H_3O, 42) and 310 (M^+-C_6H_{11}, 38).

9-(2-Methylpropan-2-yl)-oxycarbonyl-2-(1-oxohept-6-en-1-yl)-9-azabicyclo [4.2.1]non-2-ene (**115**)

Method 1: To a solution of keto vinylcarbamate (**117**) (624 mg, 2.06 mmol) in *p*-dioxane (31 ml) was added water (8 ml) and $HCl_{(aq)}$ (S.G. 1.18) (1.5 ml, ≈17 mmol). The mixture was heated to reflux, under nitrogen, for 1.75h, cooled to in ice/water and basified with 20M $NaOH_{(aq)}$. The *p*-dioxane was removed *in vacuo*, the residue poured into water (10 ml) and extracted with methylene chloride (5 x 10ml). The combined organic layers were concentrated *in vacuo*, the residue was taken up in 2:1 THF-water (40 ml), triethylamine (0.72 ml, 5.17 mmol) and di-*t*-butyldicarbonate (1.124 g, 7.29 mmol) added. After 4h at room temperature the THF was removed *in vacuo*, the residue poured into water (10 ml) and extracted with methylene chloride (5 × 10 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the *t*-butyl carbamate (**115**) as a clear colourless oil (524 mg, 76%); R_F (ethyl acetate-petroleum 1:9) = 0.24; υ_{max} (thin film) 3090 (alkene C-H), 2930br (alkane C-H), 1660br (α,β-unsaturated ketone and carbamate C=O), 1390, .1365, 1335, 1285, 1250, 1165, 1110, 1065, 1015, 995, 935, 910, 865, 835 and 775 cm^{-1}; δ_H (270MHz) 1.10-2.70 (16H, m, 8 C*H*$_2$), 1.37 (6.3H, s, 0.7 C(C*H*$_3$)$_3$), 1.44 (2.7H, s, 0.3 C(C*H*$_3$)$_3$), 4.22-4.48 (1H, m, C6*H*), 4.88-5.22 (3H, m, C=C*H*$_2$ and C1*H*), 5.70-5.89 (1H, m, C=C*H*) and 6.72-6.88 (1H, m, C3*H*); δ_C (67.8MHz), 24.2, 24.5, 28.5, 28.7, 28.8, 30.1, 30.6, 30.9, 31.7, 32.7, 33.6, 37.0, 53.4, 54.6, 55.3, 55.6, 76.6, 77.6, 79.3, 114.7, 138.5, 139.7, 140.8, 149.9, 153.2 and 200.0; m/z (E.I.) 277 (M^+-C_4H_8, 6%), 260 (M^+-C_4H_9O, 6), 233 (M^+-$C_5H_8O_2$, 7), 222 (M^+-$C_7H_{11}O$, 5) and 57 ($C_4H_9^+$, 100); m/z (C.I.) 334 (MH^+, 4%), 278 (MH^+-C_4H_8, 100) and 260 (MH^+-$C_4H_{10}O$,29).

Method 2: To a solution of 1,3-dithianyl vinylcarbamate (**114**) (22 mg, 0.056 mmol) in *p*-dioxane (10 ml) was added water (2.5 ml) and $HCl_{(aq)}$ (S.G. 1.18) (0.4 ml, ≈4.6 mmol). The mixture was heated to reflux under nitrogen for 8h then cooled in

ice/water and basified with 20M $NaOH_{(aq)}$. The *p*-dioxane was removed *in vacuo*, the residue poured into water (10 ml) and extracted with methylene chloride (5 x 5 ml). The combined organic layers were concentrated *in vacuo* and then dissolved in 2:1 THF-water (9 ml). Triethylamine (15.6 µl, 0.11 mmol) and di-*t*-butyldicarbonate (18 mg, 0.117 mmol) were added and the mixture stirred at room temperature for 16h. The THF was removed *in vacuo*, the residue poured into water (10 ml) and extracted with methylene chloride (5 x 5 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the *t*-butyl carbamate (**115**) as a clear colourless oil (7.6 mg, 42%); Physical and spectral data identical with material formed by method 1.

9-(Methyl)-2-(1-oxohept-6-en-1-yl)-9-azabicyclo[4.2.1]non-2-ene (**116**)

To a solution of alkylated 1,3-dithiane (**113**) (2.295 g, 6.80 mmol) in *p*-dioxane (100 ml) was added water (25 ml) and $HCl_{(aq)}$ (S.G. 1.18) (5 ml, ≈58 mmol). The mixture was heated to reflux under nitrogen for 20h, cooled in ice/water and basified with 20M $NaOH_{(aq)}$. The *p*-dioxane was removed *in vacuo*, the residue poured into water (10 ml) and extracted with methylene chloride (5 x 10 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave reclaimed alkylated 1,3-dithiane (**113**) (425 mg, 19%) and the α,β-unsaturated ketone (**116**) as a clear colourless oil (1.038 g, 62%); R_F (diethyl ether-methanol 4:1) = 0.36; (Found: M^+, 247.1915. $C_{16}H_{25}NO$ requires M^+, 247.1934); υ_{max} (thin film) 3090 (alkene C-H), 2930br (alkane C-H), 1660 (α,β-unsaturated ketone), 1445, 1260, 1215, 1170, 1070, 1060, 995, 960, 915, 840 and 745 cm^{-1}; δ_H (270MHz) 1.20-2.75 (16H, m, 8 CH_2), 2.31 (3H, s, N-CH_3), 3.37-3.46 ((1H, m, C6*H*), 4.44 (1H, dbr, J 9Hz, C1*H*), 4.90-5.06 (2H, m, C=CH_2), 5.70-5.88 (1H, m, C=C*H*) and 6.87-6.97 (1H, m, C3*H*); δ_C (67.8MHz) 24.4 (CH_2), 24.7 (CH_2), 25.6 (CH_2), 28.4 (CH_2), 28.5 (CH_2), 31.4 (CH_2), 33.5 (CH_2), 36.3 (N-CH_3), 36.9 (CH_2), 58.9, 62.9, 114.4 (C=CH_2), 138.5, 141.1, 148.3 (*C*2) and 201.2 (*C*10); m/z (E.I.) 247 (M^+,

41%), 164 (M^+-C_6H_{11}, 32), 136 (M^+-$C_7H_{11}O$, 100) and 83 ($C_6H_{11}^+$, 42).

2-(1-Oxohept-6-en-1-yl)-9-vinyloxycarbonyl-9-azabicyclo[4.2.1]non-2-ene (**117**)

To a solution of tertiary amine (**116**) (821 mg, 3.32 mmol) in methylene chloride (35 ml) was added vinyl chloroformate (0.44 ml, 5.18 mmol) and the mixture heated to reflux, under nitrogen and in the dark for 16h. The reaction mixture was concentrated *in vacuo*. Chromatography gave the vinyl carbamate (**117**) as a clear colourless oil (794 mg, 79%); R_F (ethyl acetate-petroleum 1:9) = 0.46; υ_{max} (thin film) 3100 (alkene C-H), 2940br (alkane C-H), 1710 (carbamate C=O), 1655 (α,β-unsaturated ketone), 1405, 1325, 1250, 1210, 1145br, 1085, 1015, 995, 950, 935, 910, 860, 835 and 755 cm^{-1}; δ_H (270MHz) 1.15-2.75 (16H, m, 8 C*H*$_2$), 4.36 (0.65H, dd, J 6.2 and 1.5Hz, 0.65 OC=C*H*$_{trans}$), 4.41 (0.35H, dd, J 6.2 and 1.5Hz, 0.35 OC=C*H*$_{trans}$), 4.45-4.55 (1H, m, C6*H*), 4.66 (0.65H, dd, J 13.9 and 1.5Hz, 0.65 OC=C*H*$_{cis}$), 4.75 (0.35H, dd, J 13.9 and 1.5Hz, 0.35 OC=C*H*$_{cis}$), 4.88-5.06 (2H, m, C=C*H*$_2$), 5.26 (0.35H, dbr, J 9Hz, 0.35 C1*H*), 5.31 (0.65H, dbr, J 9Hz, 0.65 C1*H*), 5.68-5.88 (1H, m, CC*H*=C), 6.78-6.90 (1H, m, C3*H*), 7.16 (0.65H, dd, J 13.9 and 6.2Hz, 0.65 OC*H*=C) and 7.21 (0.35H, dd, J 13.9 and 6.2Hz, 0.35 OC*H*=C); δ_C (67.8MHz) 24.1, 24.2, 24.4, 28.6, 29.7, 30.4, 31.0, 31.7, 32.2, 33.5, 37.0, 37.1, 53.6, 54.7, 55.8, 56.3, 94.9, 95.1, 114.5, 114.6, 138.5, 140.2, 141.1, 142.1, 142.5, 148.9 and 199.9; m/z (E.I.) 276 (M^+-C_2H_3, 16%), 260 (M^+-C_2H_3O, 100), 232 (M^+-$C_3H_3O_2$, 7) and 83 ($C_6H_{11}^+$, 39); m/z (C.I.) 304 (MH^+, 31%), 276 (MH^+-C_2H_4, 21) and 260 (MH^+-C_2H_4O, 100).

9-((2-Methylpropan-2-yl)-oxycarbonyl)-2-(1,6-dioxohexan-1-yl)-9-azabicyclo [4.2.1]non-2-ene (**120**)

In to a solution of primary alkene (**115**) (152 mg, 0.456 mmol) in methylene chloride (20 ml) at -78°C was bubbled aliquots of ozone in oxygen until none of alkene **115** remained by t.l.c.. Triphenylphosphine (146 mg, 0.557 mmol) was

added and the mixture allowed to slowly warm to room temperature before stirring for 2h. The reaction mixture was concentrated *in vacuo*. Chromatography gave the aldehyde (**120**) as a pale yellow oil (68 mg, 44%); R_F (ethyl acetate-petroleum 1:1) = 0.56; υ_{max} ($CHCl_3$) 2950br (alkane C-H), 2840 (alkane C-H), 2730 (aldehyde C-H), 1720 (aldehyde C=O), 1680br (carbamate C=O and α,β-unsaturated ketone), 1450, 1390, 1370, 1155, 1115, 940 and 860 cm^{-1}; δ_H (270MHz) 1.10-2.80 (25H, m, 8 C*H*$_2$ and C(C*H*$_3$)$_3$), 4.20-4.55 (1H, m, C6*H*), 5.07-5.21 (1H, m, C1*H*), 6.75-6.87 (1H, m, C3*H*) and 9.74-9.80 (1H, sbr, O=C-*H*); m/z (E.I.) 113 ($C_6H_9O_2^+$, 8%) and 57 ($C_4H_9^+$, 100); m/z (C.I.) 336 (MH^+, 2%), 142 (100) and 113 ($C_6H_9O_2^+$, 26).

9-((2-Methylpropan-2-yl)-oxycarbonyl)-2-(7(2-methylpropan-2-yl)oxycarbonyl)-1-oxo-7-azatridecan-1-yl)-9-azabicyclo[4.2.1]non-2-ene (**127**)

To a solution of aldehyde (**120**) (13 mg, 38.8 μmol) in methanol (1 ml) was added n-hexylamine (31 μl, 0.22 mmol) and 2.4M HCl in methanol (33μl, 79 mmol). Sodium cyanoborohydride (1.7 mg, 27μmol) was added and the mixture stirred at room temperature, under nitrogen for 1h. The reaction mixture was concentrated *in vacuo*, the residue added to water (10 ml) and made basic with dilute $NaOH_{(aq)}$. The aqueous mixture was extracted with methylene chloride (5 x 5 ml). The methylene chloride was removed *in vacuo*, the residue taken up in 2:1 THF-water (2 ml) several equivalents of di-*t*-butyldicarbonate and triethylamine added. After 16h the THF was removed *in vacuo*, the residue poured into water (5 ml) and extracted with methylene chloride (5 x 3 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the bis-*t*-butyl carbamate (**127**) as a colourless glass (2.6 mg, 13%); R_F (ethyl acetate-petroleum 1:4) = 0.44; υ_{max} ($CHCl_3$) 2930br (alkane C-H), 2870 (alkane C-H), 1665br (carbamate C=O and α,β-unsaturated ketone), 1445, 1405, 1370, 1155, 1115, 1045, 1005, 940 and 860 cm^{-1}; δ_H (270MHz) 1.00-2.70 (45H, m, 12 C*H*$_2$ and 7 C*H*$_3$), 3.00-3.15 (4H, m, 2 CONC*H*), 4.05-4.20 (1H, m, C6*H*), 5.00-5.15 (1H, m, C1*H*) and 6.65-6.80 (1H, m,

C3*H*); m/z (E.I.) 420 (M^+-(C_4H_8 + CO_2), 1%), 419 (M^+-Boc, 1), 363 (M^+-(Boc + C_4H_8), 2) and 57 ($C_4H_9^+$, 100); m/z (C.I.) 521 (MH^+, 1%), 421 (MH^+- (C_4H_8 + CO_2), 26), 365 (MH^+-(2 C_4H_8 + CO_2), 25) and 214 ($C_{12}H_{24}NO_2^+$, 100).

2-(1-Methoxycarbonyl-6-oxohexan-6-yl)-9-(2-methylpropan-2-yl)-oxycarbonyl-9-azabicyclo[4.2.1]non-2-en (**128**)

To a solution of aldehyde (**120**) (41 mg, 0.122 mmol) in 9:1 methanol-water (2 ml) was added $NaHCO_3$ (195 mg, 2.32 mmol) and 1.55M bromine in 9:1 methanol-water (0.31 ml, 0.48 mmol). After 30min $Na_2S_2O_{3(aq)}$ was added, the methanol removed *in vacuo* and the residue poured into water (10 ml). The aqueous mixture was extracted with methylene chloride (5 x 4 ml), the combined extracts dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the methyl ester (**128**) as an opaque colourless glass (11 mg, 25%); R_F (diethyl ether-petroleum 3:2) = 0.45; υ_{max} (thin film) 2960br (alkane C-H), 1735 (ester C=O), 1685br (carbamate C=O and α;β-unsaturated ketone), 1435, 1395, 1365, 1340, 1250, 1170, 1110, 1065, 1015, 995, 935, 865, 835 and 775 cm^{-1}; δ_H (270MHz) 1.00-2.90 (25H, m, 8 C*H*$_2$ and C(C*H*$_3$)$_3$), 3.66 (3H, s, CO_2CH_3), 4.18-4.50 (1H, m, C6*H*), 5.06-5.20 (1H, m, C1*H*) and 6.72-6.88 (1H, m, C3*H*); m/z (E.I.) 365 (M^+, 1%), 309 (M^+-C_4H_8, 2), 306 (M^+-CO_2CH_3, 1), 265 (M^+-(C_4H_8 + CO_2), 12) and 57 ($C_4H_9^+$, 100); m/z (C.I.) 366 (MH^+, 3%), 310 (MH^+-C_4H_8, 46), 265 (MH^+-Boc, 38) and 143 ($C_7H_{11}O_3^+$, 100).

2-(1-Methoxycarbonyl-6-oxohexan-1-yl)-9-azabicyclo[4.2.1]non-2-ene hydrochloride (**129**)

A solution of *t*-butyl carbamate (**128**) (3.5 mg, 9.6 μmol) in trifluoroacetic acid was stirred at 0°C under nitrogen for 1h. The trifluoroacetic acid was removed *in vacuo*, the residue taken up in diethyl ether and saturated with dry $HCl_{(g)}$ before removing the diethyl ether *in vacuo*, fresh diethyl ether was added and the process repeated

twice. Chromatography gave the secondary amine hydrochloride (**129**) as an opaque colourless glass (2.9 mg, 100%); R_F (methanol-diethyl ether 2:3) = 0.22; (Found: M^+, 265.1655. $C_{15}H_{23}NO_3$ requires 265.1675); υ_{max} ($CHCl_3$) 2940br (alkane C-H), 1730 (ester), 1670 (α,β-unsaturated ketone), 1605, 1590, 1425, 1400, 1380, 1365, 1235, 1165, 1145, 1015, 935 and 810 cm^{-1}; δ_H (270MHz) 1.15-2.90 (16H, m, 8 C*H*$_2$), 3.66 (3H, s, CO_2CH_3), 4.28-4.40 (1H, sbr, C6*H*), 5.23 (1H, dbr, J 9Hz, C1*H*), 7.09-7.18 (1H, m, C3*H*), 9.10-9.50 (1H, sbr, N^+-*H*) and 9.80-10.30 (1H, sbr, N^+-*H*); m/z (E.I.) 265 (M^+, 46%), 234 (M^+-OCH_3, 30), 192 (M^+-$C_3H_5O_2$, 10), 178 (M^+-$C_4H_7O_2$, 19), 164 (M^+-$C_5H_9O_2$, 33), 150 (M^+-$C_6H_{11}O_2$, 72), 122 (M^+-$C_7H_{11}O_3$, 80) and 69 ($C_4H_5O^+$, 100).

2-(*N*-Hexyladipamoyl)-9-(2-methylpropan-2-yl)-oxycarbonyl-9-azabicyclo [4.2.1]non-2-ene (**130**)

To a solution of ester (**128**) (6 mg, 16.4 μmol) in THF (1 ml) was added a solution of lithium hydroxide (0.7 mg, 29 μmol) in water (0.15 ml) with stirring. After 6h the THF was removed *in vacuo*, the residue poured into water (5 ml) and acidified to pH2 with dilute $HCl_{(aq)}$ before extracting with methylene chloride (5 x 4 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. The crude acid was redissolved in methylene chloride (1 ml) and n-hexylamine (2.5 μl, 19 μmol) and *N,N*-dicyclohexylcarbodiimide (5.8 mg, 28 μmol) in methylene chloride (1 ml) added at 0°C. The reaction mixture was allowed to warm slowly to room temperature and stirred for 16h before removing the methylene chloride *in vacuo*. Chromatography gave the secondary amide (**130**) as a clear colourless glass (4.0 mg, 56%); R_F (diethyl ether-petroleum 3:2) = 0.11; υ_{max} ($CHCl_3$) 3490 (N-H), 3370 (N-H), 2980br (alkane C-H), 2910 (alkane C-H), 1695br (amide C=O, carbamate C=O, and α,β-unsaturated ketone), 1520, 1415, 1390, 1185, 1135, 1035, 955 and 915 cm^{-1}; δ_H (270MHz) 0.80-2.80 (36H, m, 12 C*H*$_2$ and 4 C*H*$_3$), 3.57-3.77 (1H, m, 0.5 C(O)NC*H*$_2$), 3.82-4.00 (1H, m, 0.5 C(O)NC*H*$_2$), 4.25-4.47 (1H, m, C6*H*),

5.08-5.18 (1H, m, C1*H*) and 6.75-6.88 (1H, m, C3*H*).

2-(*N*-Hexyladipamoyl)-9-azabicyclo[4.2.1]non-2-ene hydrochloride(**131**)

A solution of *t*-butyl carbamate (**130**) (3.1 mg, 7.1 μmol) in trifluoroacetic acid (1 ml) was stirred at 0°C under nitrogen for 10min. The trifluoroacetic acid was removed *in vacuo*, the residue taken up in diethyl ether and saturated with dry $HCl_{(g)}$. The diethyl ether was removed *in vacuo*, fresh diethyl ether added and the process repeated twice. Chromatography gave the secondary amine hydrochloride (**131**) as a colourless glass (0.7 mg, 26%); R_F (methanol-methylene chloride 1:9) = 0.29; δ_H (270MHz) 0.80-2.95 (27H, m, 12 CH_2 and CH_3), 3.59-3.77 (1H, m, 0.5 C(O)NCH_2), 3.85-4.03 (1H, m, 0.5 C(O)NCH_2), 4.27-4.38 (1H, m, C6*H*), 5.14-5.26 (1H, m, C1*H*) 7.07-7.17 (1H, m, C3*H*), 8.65-8.90 (1H, sbr, N^+-*H*) and 10.00-10.30 (1H, sbr, N^+-*H*).

2-(2((4-Bromophenyl)-methyl)-1,3-dithian-2-yl)-9-methyl-9-azabicyclo [4.2.1]non-2-ene (**139**)

The anion of ketene-*S*,*S*-acetal **77** was formed (as described for compound **78**) using **77** (256 mg, 1.00 mmol) in THF (5 ml) and 1.6M Bu^nLi in hexane (0.75 ml, 1.2 mmol). 4-Bromobenzyl bromide (**138**)(250 mg, 1.00 mmol) in THF (3 ml) was added at -78°C. After 30min saturated $NH_4Cl_{(aq)}$ (2 ml) was added, the reaction mixture poured into water (20 ml) and extracted with methylene chloride (5 x 5 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the alkylated 1,3-dithiane (**139**) as a colourless solid which could be further purified by recrystallisation from ethyl acetate (307 mg, 72%), m.p. 151.5-152.0°C (ethyl acetate); (Found: C, 56.5; H, 6.2; N, 3.3 $C_{20}H_{26}BrNS_2$ requires C, 56.6; H, 6.2; N, 3.3%); R_F (diethyl ether-methanol 4:1) = 0.17; υ_{max} ($CHCl_3$) 2930br (alkane C-H), 1480, 1425, 1150, 1125, 1095, 1070, 1010, 955, 935, 915 and 885 cm^{-1}; δ_H (270MHz) 1.42-2.98 (14H, m, 7 OCH_2), 2.44 (3H, s, N-CH_3),

3.06 (2H, q, J 13.2Hz, Ar-C*H*$_2$), 3.45 (1H, sbr, C6*H*), 4.07 (1H, dbr, J 9Hz, C1*H*), 5.98-6.12 (1H, m, C3*H*) and 7.34-7.45 (4H, m, aromatic); m/z (E.I.) 425 (M(^{81}Br)$^+$ 23%), 423 (M(^{79}Br)$^+$, 22), 319 (M(^{81}Br)$^+$-$C_3H_6S_2$, 12), 317 ((^{79}Br)$^+$-$C_3H_6S_2$, 12), 254 (M^+-C_7H_6Br, 84) and 82 ($C_5H_8N^+$, 100).

2-(2-(4-Bromophenyl)-oxoethan-1-yl)-9-methyl-9-azabicyclo[4.2.1]non-2-ene (**140**)

To a solution of alkylated 1,3-dithiane (**139**) (91.2 mg, 0.215 mmol) in *p*-dioxane (8 ml) was added water (2.4 ml) and $HCl_{(aq)}$ (S.G. 1.18) (0.8 ml, ≈9.3 mmol). The mixture was heated to reflux under nitrogen for 30h then cooled in ice/water and basified with 20M $NaOH_{(aq)}$. The *p*-dioxane was removed *in vacuo*, the residue poured into water (10 ml) and extracted with methylene chloride (5 x 5 ml). The combined extracts were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave unhydrolysed 1,3-dithiane (**139**) (24.7 mg, 27%) and the α,β-unsaturated ketone (**140**) as a colourless solid which could be further purified by trituration with petroleum-diethyl ether (47.3 mg, 66%), m.p. 83-83.5°C (diethyl ether-petroleum); R_F (diethyl ether-methanol 4:1) = 0.16; (Found: C, 61.0; H, 6.1; N, 4.1. $C_{17}H_{20}BrNO$ requires C, 6.11; H, 6.0; N, 4.2%); υ_{max} ($CHCl_3$) 2935br (alkane C-H), 1660 (α,β-unsaturated ketone), 1635, 1490, 1435, 1350, 1315, 1160, 1080, 1015 and 940 cm^{-1}; δ_H (270MHz) 1.34-2.55 (8H, m, 4 C*H*$_2$), 2.29 (3H, s, N-C*H*$_3$), 3.38-3.49 (1H, m, C6*H*), 3.92 (2H, s, COC*H*$_2$-Ar), 4.44 (1H, dbr, J 9Hz, C1*H*) and 6.98-7.48 (5H, m, C3*H* and aromatic); m/z (E.I.) 335 (M(^{81}Br)$^+$, 48%), 333 (M(^{79}Br)$^+$, 46), 171 ($C_7H_6{}^{81}Br^+$, 12), 169 ($C_7H_6{}^{79}Br^+$, 12), 164 (M^+-C_7H_6Br, 100) and 136 (M^+-C_8H_6BrO, 98).

2-(2-((4-Iodophenyl)-methyl)-1,3-dithian-2-yl)-9-methyl-9-azabicyclo [4.2.1]non-2-ene (**142**)

The anion of ketene-S,S-acetal **77** was formed (as described for compound **78**) using **77** (264 mg, 1.03 mmol) in THF (5 ml) and 1.6M Bu^{n}Li in hexane (0.75 ml, 1.20

mmol). 4-Iodobenzyl bromide (**141**)(307 mg, 1.03 mmol) in THF (2 ml) was added at -78°C. After 30min saturated $NH_4Cl_{(aq)}$ (2 ml) was added, the reaction mixture poured into water (20 ml) and extracted with methylene chloride (5 x 5 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave the alkylated 1,3-dithiane (**142**) as a colourless solid which could be further purified by recrystallisation from ethyl acetate (384 mg, 79%), m.p. 147-147.5°C (ethyl acetate); R_F (diethyl ether-methanol 4:1) = 0.55; (Found: C, 50.7; H, 5.5; N, 2.9. $C_{20}H_{26}INS_2$ requires C, 51.0; H, 5.6 N, 3.0%); υ_{max} (thin film) 3040 and 3020 (aryl and alkene C-H), 2920br (alkane C-H), 1485, 1430, 1265, 1200, 1120, 1095, 1065, 1005, 910, 890, 835, 800, 770, 735 and 705 cm^{-1}; δ_H (270MHz) 1.40-3.10 (14H, m, 7 C*H*$_2$), 2.47 (3H, s, N-C*H*$_3$), 3.04 (2H, q, J 14Hz, Ar-C*H*$_2$), 3.40-3.60 (1H, s br, C6*H*), 4.00-4.20 (1H, s br, C1*H*), 6.00-6.20 (1H, s br, C3*H*) and 7.22-7.62 (4H, m, aromatic); m/z (E.I.) 471 (M^+, 40%), 364 (M^+-$C_3H_7S_2$, 44), 254 (M^+-C_7H_7I, 98), 217 ($C_7H_6I^+$, 14) and 82 ($C_5H_8N^+$, 100).

2-(2-(4-Iodophenyl)-1-oxoethan-1-yl)-9-methyl-9-azabicyclo[4.2.1]non-2-ene (**143**)

To a solution of alkylated 1,3-dithiane (**142**) (239 mg, 0.507 mmol) in *p*-dioxane (20 ml) was added water (5 ml) and HCl (S.G. 1.18) (2 ml, ≈23 mmol). The mixture was heated to reflux under nitrogen for 16h then cooled *in* ice/water and basified with 20M $NaOH_{(aq)}$. The *p*-dioxane was removed *in vacuo,* the residue poured into water (25 ml) and extracted with methylene chloride (5 x 10 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave unhydrolysed 1,3-dithiane (**142**) (44 mg, 18%) and the α,β-unsaturated ketone (**143**) as a colourless solid which could be further purified by trituration with petroleum-diethyl ether (149 mg, 77%), m.p.120-120.5°C (diethyl ether-petroleum); (Found: C, 53.3; H, 5.35; N, 3.6. $C_{17}H_{20}NIO$ requires C, 53.6; H, 5.3; N, 3.7%); υ_{max} (thin film) 3040 (aryl and alkene C-H), 2930br (alkane C-H), 1665 (α,β-unsaturated ketone), 1625 (aryl), 1485, 1380, 1340, 1315, 1255, 1215, 1180,

1060, 1010, 945, 915, 840, 795 and 730 cm^{-1}; δ_H (270MHz) 1.00-2.60 (8H, m, 4 C*H*$_2$), 2.27 (3H, s, N-C*H*$_3$), 3.35-3.50 (1H, m, C6*H*), 3.89 (2H, s, COC*H*$_2$-Ar), 4.42 (1H, dbr, J 9Hz, C1*H*), 6.86-7.68 (4H, m, aromatic) and 6.98-7.08 (1H, m, C6*H*); m/z (E.I.) 381 (M^+, 94%), 217 ($C_7H_6I^+$, 20), 164 (M^+-C_7H_6I, 93) and 136 (M^+-C_8H_6IO, 100).

9-(2-Methylpropan-2-yl)-2-((propan-1-oxo-1-yl)-oxycarbonyl)-9-azabicylo [4.2.1]non-2-ene (**149**)

To a solution of methyl ketone (**148**) (41 mg, 0.155 mmol) in THF (3 ml) was added LDA (0.19 mmol) in THF (0.8 ml) at 0°C under nitrogen. After 1h iodomethane (26 mg, 0.183 mmol) in THF (0.3 ml) was added and the mixture stirred at 0°C for 2.5h. Saturated $NH_4Cl_{(aq)}$ (1 ml) was added, the mixture poured into water (10 ml) and extracted with methylene chloride (5 x 5 ml). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo*. Chromatography gave reclaimed methyl ketone (**148**) (8 mg, 20%) and the desired ethyl ketone (**149**) as a clear colourless oil (14 mg, 32%); R_F (diethyl ether-petroleum) = 0.60; υ_{max} (thin film) 2980 (alkane C-H) (C-H), 2940 (alkane C-H), 1670br (carbamate C=O and α,β-unsaturated ketone), 1395br, 1365, 1340, 1250, 1175br, 1110, 1070, 1015, 940, 925, 870, 840 and 775 cm^{-1}; δ_H (270MHz) 1.03-1.16 (3H, m, $COCH_2CH_3$), 1.37 (6.3H, s, 0.7 C(C*H*$_3$)$_3$), 1.44 (2.7H, s, 0.3 C(C*H*$_3$)$_3$), 1.54-2.51 (8H, m, 4 C*H*$_2$), 2.52-2.79 (2H, m, COC*H*$_2$CH$_3$), 4.22-4.49 (1H, m, C6*H*), 5.06-5.24 (1H, m, C1*H*) and 6.74-6.88 (1H, m, C3*H*); m/z (E.I.) 279 (M^+, 4%), 223 (M^+-C_4H_8, 12), 206 (M^+-C_4H_90, 10), 194 (M^+-(C_4H_8 + C_2H_5), 16), 179 (M^+-(C_4H_8 + CO_2), 50), 150 (M^+-(C_4H_8 + CO_2 + C_2H_5), 47) and 57 ($C_4H_9^+$, 100).

2-(Propan-1-oxo-1-yl)-9-azabicylo[4.2.1]non-2-ene hydrochloride(**150**)

A solution of *t*-butylcarbamate (**149**) (13 mg, 47μmol) in trifluoroacetic acid (1 ml) was stirred at 0°C for 15min under nitrogen. The trifluoroacetic acid was removed

in vacuo, the residue taken up in diethyl ether and saturated with dry $HCl_{(g)}$. The solvent was removed *in vacuo*, fresh diethyl ether added and the process repeated twice. Chromatography gave the secondary amine hydrochloride (**150**) as a clear colourless glass (6 mg, 60%); R_F (diethyl ether-methanol) = 0.15; (Found M^+, 179.1301. $C_{11}H_{17}NO$ requires M^+, 179.1309); υ_{max} (thin film) 3730-3150 (N-H), 2920br (alkane C-H), 1665 (α,β-unsaturated ketone), 1590, 1460, 1405, 1370, 1295, 1125, 1080, 1020, 960, 910, 840, 810 and 735 cm^{-1}; δ_H (270MHz) 1.10 (3H, t, J 7.2Hz, $COCH_2CH_3$), 1.20-2.88 (10H, m, 4 CH_2 and $COCH_2CH_3$), 4.26-4.43 (1H, m, C6*H*), 5.23 (1H, dbr, J 8.8Hz, C1*H*) and 7.09-7.19 (1H, m, C3*H*); m/z (E.I.) 179 (M^+, 34%), 150 (M^+-C_2H_5, 39), 122 (M^+-C_3H_5O, 34) and 57 ($C_3H_5O^+$, 100).

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